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OCTOBER 1952

VOLUME 90 NUMBER 4

Published Monthly by

AMERICAN MEDICAL ASSOCIATION

535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 23, 1909, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $10.00

TABLE OF CONTENTS FIRST PAGE

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CONTENTS

Original Articles

PAGE

Hypochloremic Alkalosis Induced by Mercurial Diuretics in Congestive Heart Failure
John F. Stapleton, M.D., and W. Proctor Harvey, M.D., Washington, D. C. 425

Controlled Hypoxemia Test for Coronary Insufficiency Employing the Millikan Oximeter
Howard E. Tewell Jr., M.D., and J. Stuart Pritchard, M.D., Chicago 435

Residual Effects of Rickettsial Disease on the Central Nervous System
Marvin J. Rosenblum, M.D.; Richard L. Masland, M.D., and George T. Harrell, M.D., Winston-Salem, N. C. 444

Histoplasmosis
Henry Pinkerton, M.D., St. Louis, and Lalla Iverson, M.D., Washington, D. C. 456

Acute Diffuse Interstitial Fibrosis of the Lungs
William P. Callahan Jr., M.D.; John C. Sutherland, M.D.; John K. Fulton, M.D., and John R. Kline, M.D., Wichita, Kan. 468

Porphyria Cutanea Tarda
Alastair G. Macgregor, M.D., M.R.C.P., Sheffield, England, and R. E. H. Nicholas, Ph.D., A.R.I.C., and C. Rimington, Ph.D., D.Sc., London, England 483

Observations on Alleged Serotonin- (Enteramine)-like Nature of Cerebral Pressor Substance of Taylor, Page, and Corcoran
Vittorio Erspamer, M.D., Bari, Italy 505

Progress in Internal Medicine

Recent Advances in Nutrition and Metabolism
Grace A. Goldsmith, M.D.; Walter G. Unglaub, M.D., and Janis Gibbens, M.S., New Orleans 513

Case Reports

Diphtheroid Subacute Bacterial Endocarditis Successfully Treated with Bacitracin
Joshua F. Zendel, M.D., and Alfred Lubart, M.D., New York 562

Asymptomatic Arteriovenous Fistula of the Lung
Theodore J. Talbot, M.D., and Jacob J. Silverman, M.D., Staten Island, N. Y. 569

Regular Departments

News and Comment 575

Books 576

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Manuscripts for publication, books for review and correspondence relating to the editorial management should be sent to the Chief Editor, Dr. Paul S. Rhoads, 535 North Dearborn Street, Chicago 10. Communications regarding subscriptions, reprints, etc., should be addressed, A. M. A. ARCHIVES OF INTERNAL MEDICINE, American Medical Association, 535 North Dearborn Street, Chicago 10.

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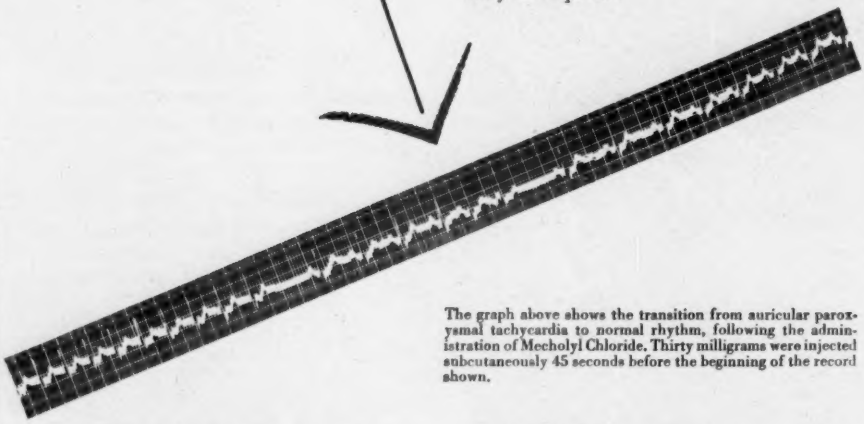

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HYPOCHLOREMIC ALKALOSIS INDUCED BY MERCURIAL DIURETICS IN CONGESTIVE HEART FAILURE

A Reversible Form of So-Called Refractory Heart Failure

JOHN F. STAPLETON, M.D.
AND
W. PROCTOR HARVEY, M.D.
WASHINGTON, D. C.

CONGESTIVE heart failure that no longer responds with diuresis to mercurial injections usually carries a grave prognosis, often leading to progressive cardiac decompensation and death. This condition represents a real challenge to the practicing physician. It has become evident that a host of factors may be responsible for the development of this refractoriness. The irreversible trend of events in some cases turns out to be due to an unsuspected complication, such as myocardial infarction, pulmonary infarction, renal insufficiency, pneumonia, active rheumatic carditis, cerebral vascular accident, anemia, hyperthyroidism, infection, arrhythmias, or others.[1] Occasionally, inadequate digitalization or insufficient sodium restriction may be responsible. However, a group of patients with so-called refractory heart failure continues a downhill course in the absence of these complications. It is becoming increasingly clear that this group ceases to respond to mercurials as a result of an electrolyte imbalance, the nature of which is variable and the reasons for the development of which are not altogether clear. Restoration of normal electrolyte balance, however, has been associated with renewed response to mercury. This response is often dramatic, occurring as it does in a patient who appeared to have irreversible heart failure.

The low-salt syndrome has received considerable attention recently as one form of a postdiuresis electrolyte disturbance.[2] This syndrome, characterized by weakness, nausea, apathy, and sometimes mental aberration, is successfully treated with hypertonic saline solution given either orally or parenterally. The results may be

Dr. Stapleton is Research Fellow in Medicine, and Dr. Harvey is Assistant Professor of Medicine.

From the Department of Medicine, Georgetown University Hospital and Georgetown University School of Medicine.

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highly gratifying. A common electrolyte pattern in the low-salt syndrome consists of low serum sodium, low serum chloride, and low carbon-dioxide-combining power, with acidosis and azotemia.

However, another form of acid-base disturbance following mercurial diuresis has received less attention in the literature. This is the hypochloremic alkalosis syndrome which has recently been stressed by Schwartz.[3] It is of considerable importance in that it is surprisingly common, simply diagnosed and readily treated. The pattern of this syndrome, in contrast to that of the low-salt syndrome, consists of low serum chloride and elevated carbon-dioxide-combining power, with alkalosis. The serum sodium is usually normal. These patients respond well to administration of large doses of chloride in a form of acid salts, such as ammonium chloride, calcium chloride, or diluted hydrochloric acid. Of these, ammonium chloride is the most commonly used. Administration of hypertonic saline solution would not appear indicated in this group.

In a review of cases that present the problem of postdiuretic hypochloremic alkalosis, an impressive similarity in the chain of events becomes apparent. The sequence usually runs about as follows: The patient often is one who has been responding well to a cardiac regimen consisting of digitalis, salt restriction, and periodic mercurial injection. A stage is reached in which this therapy fails to prevent fluid retention and the signs of congestive failure become severer. When this occurs in the absence of any complication, various "tricks" are usually tried. A change of mercurial compound may be resorted to, usually without success, or a different route of administration is tried, sometimes with good results. Although intramuscular administration is usually efficacious, at times changing to the intravenous route brings about diuresis. A change from oral or rectal administration to a parenteral route may also be effective. Aminophylline, given in adequate dosage concomitantly with and for several days following mercurial injection, has at times produced considerable diuresis in previously unresponsive patients.[4] Should these measures fail, the dose and frequency of mercurial injection are increased. The individual dose is usually kept at 1 to 2 cc. but may be raised to higher levels. The frequency of injection is also increased, many patients receiving daily injections. Ammonium chloride in varying dosage may or may not be an adjuvant in therapy. The patient becomes progressively more waterlogged and incapacitated. A stage is reached at which his condition is sometimes tagged as "irreversible myocardial insufficiency" or as "refractory," and therapy is directed toward easing his terminal downhill course. It is readily understood how one would adopt a hopeless outlook for such a patient.

When one is confronted with this type of case, a search for a disturbance in electrolytes may reveal low serum chloride and normal or elevated carbon-dioxide-combining power. Correction of these abnormalities can be lifesaving.

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It is the purpose of this paper to stress the syndrome of hypochloremic alkalosis with a report on four patients treated recently in Georgetown University Hospital.

REPORT OF CASES

CASE 1.—A 65-year-old white man with hypertension and coronary arteriosclerosis was admitted to the hospital with massive peripheral edema and severe orthopnea. Congestive failure of varying severity had been present for about three and one-half years.

For six months prior to admission mercurial injections had been administered every second or third day. Ammonium chloride had been administered in the dosage of 3 gm. every third day.

At first there was improvement with this therapy. A month prior to admission, however, it became evident that he was no longer responding. His dyspnea grew worse, and the edema spread into his thighs, genitalia, and lower torso. The patient was brought to the hospital in a moribund state, cyanotic and gasping for breath.

Physical examination disclosed the findings of severe congestive heart failure, including hydrothorax, ascites, and massive peripheral edema with involvement of the abdominal wall and genitalia.

The history pointed to a strong likelihood of hypochloremic alkalosis. A serum chloride value [5] of 99.2 mEq. per liter did not rule out this impression, and consequently the mercurial injections were discontinued. An attempt was made to increase extracellular chloride stores. Treatment was started with 8 gm. of ammonium chloride daily, and four days later he was also given 4 cc. of 10% hydrochloric acid solution (diluted in a glass of water) four times a day. The serum chloride rose to 111 mEq. per liter on the fourth day. On the morning of the sixth day of high chloride intake, he received 2 cc. of meralluride (mercuhydrin®) intramuscularly. A massive diuresis occurred. On the day of the injection the patient passed 7,000 cc. plus an unrecorded quantity which ran out around the catheter and soaked his bedsheet (Fig. 1).

The diuresis lasted for several days, during which time he was given large quantities of water and orange juice (given for potassium content) by mouth. He felt weak and "washed out" but breathed more easily. The high oral ammonium chloride intake was continued.

Five days after the first injection, another 2 cc. of meralluride was administered; again a considerable diuresis occurred. The patient's weight curve dropped to a low of 120 lb. (54.4 kg.) after the second injection, this representing a 33-lb. (15-kg.) loss over the admission weight. The patient was breathing comfortably, and his edema had subsided except for some residual edema of the genitalia. A chest roentgenogram disclosed improvement, although some pleural fluid persisted (Fig. 2). Clinical improvement was impressive, and the patient was discharged. He did well as an outpatient for more than 10 months, then died unexpectedly.

Comment on Case 1.—This patient was admitted to the hospital as being in a hopeless, terminal condition. He had been receiving mercurials without response two to three times weekly for months. The dose of ammonium chloride was apparently not sufficient to replace the chloride lost by the frequent use of mercurials. Although his initial serum chloride value was in the low normal range, his remarkable response to replenished chloride stores, while sodium restriction was maintained, leaves little doubt as to the cause of his refractoriness. This illustrates the individual variation in what constitutes a basal chloride level, since other patients with chloride levels of 98 mEq. per liter have had satisfactory diuresis following use of mercurials.

The transformation from a terminal condition to a controllable one was, needless to say, gratifying. In view of the possibility of acidosis occurring with ammonium chloride therapy, particularly in the presence of any renal insufficiency, he was maintained with intermittent rather than constant ammonium chloride administration.

CASE 2.—A 43-year-old white woman was admitted to the hospital in advanced congestive failure. She was known to have had mitral stenosis for at least 20 years and varying degrees of decompensation for 10 years.

Her congestive failure had become incapacitating in the three months prior to admission, even though she was receiving 6 gm. of ammonium chloride daily and 2 cc. of mercaptomerin (thiomerin®) sodium every other day.

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5. Normal values for this laboratory are as follows: sodium, 138 to 143 mEq. per liter; carbon-dioxide-combining power, 45 to 50 vol. %, and chloride, 99 to 106 mEq. per liter.

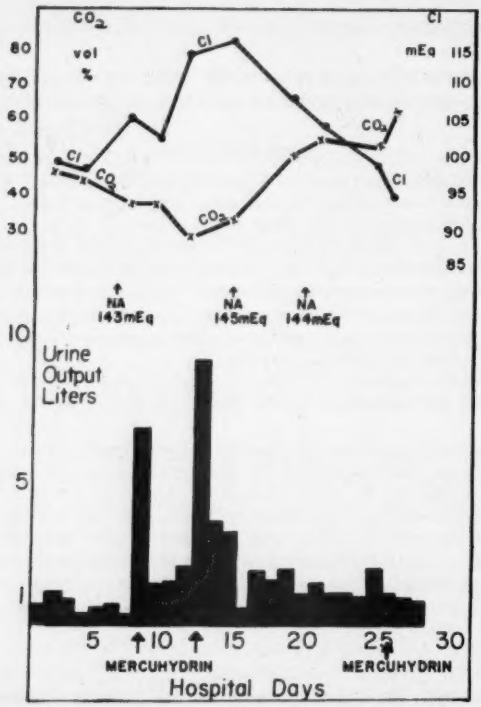

Fig. 1 (Case 1).—Chart showing electrolytic changes in 65-year-old white man with hypertensive cardiovascular disease and severe, progressive congestive failure in spite of frequent injections of meralluride (mercuhydrin®). Response was restored with only moderate increase of serum chloride. Note normal sodium value and rapid depletion of chlorides with diuresis.

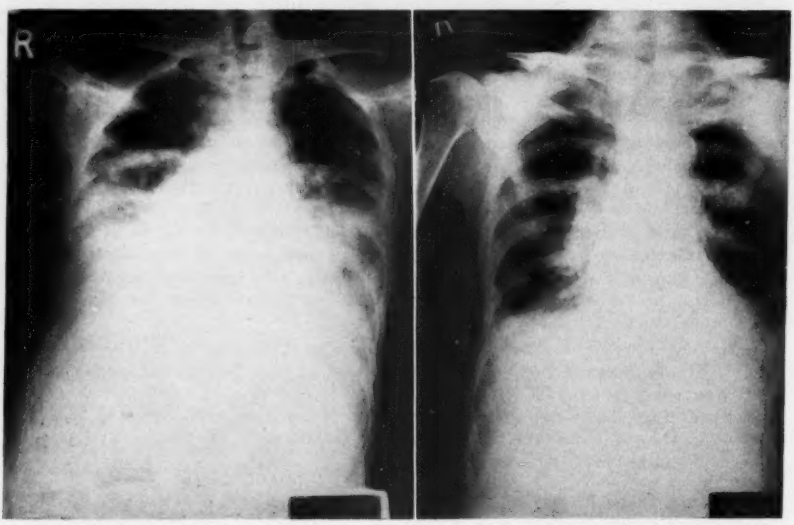

Fig. 2 (Case 1).—Roentgenograms of patient whose chart is shown in Figure 1. Note clearing of pulmonary congestion and reduction of heart size following restoration of diuretic response to mercurials.

Physical examination disclosed symptoms of advanced congestive heart failure, including hydrothorax, ascites, and signs of tricuspid valve insufficiency.

On admission, the history of steadily progressing fluid accumulation during frequent administration of doses of mercaptomerin suggested the syndrome of hypochloremic alkalosis, although she had been receiving 6.0 gm. of ammonium chloride daily. This was confirmed by the serum chloride level of 85.5 mEq. per liter. Mercaptomerin administration was stopped, and ammonium chloride was given in doses of 2 gm. four times daily. Added to this was 1 teaspoon (5 cc.) of 10% hydrochloric acid solution diluted in a glass of water three times daily. The blood chloride rose to 105 mEq. per liter in six days. At this time 2 cc. of mercaptomerin given

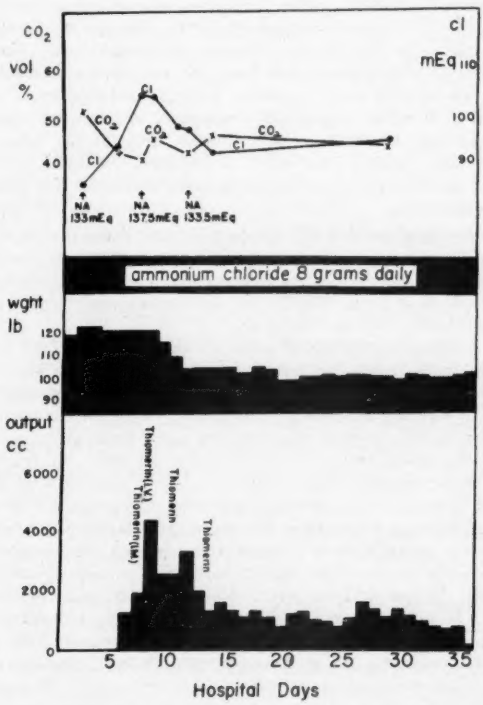

Fig. 3 (Case 2).—Chart showing electrolyte changes during therapy in 43-year-old white woman with mitral stenosis, severe decompensation, and refractoriness to mercurials. Low serum sodium value is seen. Response to mercurials (mercaptomerin [thiomerin®]) was restored, however, by administration of large doses of ammonium chloride and dilute hydrochloric acid while sodium restriction was maintained.

intramuscularly was ineffectual. On the next day 2 cc. of mercaptomerin was given intravenously. A striking diuresis resulted. Over a 72-hour period she lost 9,200 cc. of urine and 11 lb. (5 kg.) in weight (Fig. 3). Her high chloride intake was continued, and she received another intravenous injection of mercaptomerin. It should be noted that at this time her serum chloride was 97 mEq. per liter, but again a good diuresis occurred, and 6 lb. (2.7 kg.) more was lost in 24 hours. Associated with this diuresis were a reduction in peripheral edema and improvement in the patient's comfort. Hydrothorax and ascites remained relatively unchanged.

After the second diuresis, serum chloride fell to 92 mEq. per liter, at which time an intravenous mercaptomerin injection was ineffectual.

She maintained a constant low weight for about six weeks following her diuresis, at the same time continuing high chloride intake. During the early part of this six-week period, two mercaptomerin injections of 2 cc. each were given without any increase in urinary output, although the serum chloride remained elevated. The possible reason for this will be discussed below.

After six weeks of maintenance in a fairly stable state of compensation, it became evident that she was slowly reaccumulating fluid. Mercaptomerin was again given intravenously in a dose of 2 cc., resulting in another copious diuresis. The patient lost 7 lb. (3.2 kg.), with a corresponding diminution of peripheral edema. The serum chloride was then 96.9 mEq. per liter. It was felt at this time that the patient had achieved maximum hospital benefit, and thus she was discharged for outpatient care.

Comment on Case 2.—The original chloride level of 85 mEq. per liter is a striking illustration of individual variation in chloride loss following mercurial-induced diuresis. In spite of administration of 6 gm. of ammonium chloride daily, her chloride output evidently exceeded her chloride intake, and low chloride levels appeared. After her chloride stores were replenished, it took but two diureses to render her condition refractory to mercurials again. Later in the course of her hospital stay, she failed to have diuresis, although her serum chlorides were normal. Her weight was almost at "dry level" at this time, but ascitic fluid was present, a situation which is known to be often resistant to mercurial injection. Her weight fell promptly after an abdominal paracentesis.

Case 3.—A 48-year-old white man with hypertension was hospitalized during the course of an alcoholic episode. Decompensation had occurred one year previously but was readily controlled without subsequent exacerbation. An examination on admission revealed acute alcoholic intoxication. The blood pressure was 160/120. No signs of heart failure were present. Therapy in the hospital included daily mercurial injections.

During the first week of hospitalization severe congestive heart failure developed, characterized by orthopnea, gallop rhythm, pulmonary rales, and 4+ pretibial and pedal edema. His condition continued to grow worse, and he gained weight in spite of a gradual increase in daily mercurial injections to 4 cc. per injection.

At this time hypochloremic alkalosis was suspected, and a serum chloride determination was therefore obtained. The value proved to be 85.5 mEq. per liter. At this point ammonium chloride was added to the regimen. At first this was given in doses of 4 gm. daily, then 6 gm. daily. He continued to receive daily mercurial injections during this period, with no diuresis. On the third day of high chloride intake, the serum chloride reached a level of 92.3 mEq. per liter. At this time a marked diuresis occurred with a 9-lb. (4.1-kg.) weight loss in 24 hours.

Mercurial injections were then discontinued. Ammonium chloride administration was continued at 6 gm. daily. In four days the serum chloride was 102 mEq. per liter. At this time 2 cc. of meralluride produced a diuresis, with a resulting 8½-lb. (3.9-kg.) weight loss in 24 hours. Intermittent mercurial injections were continued in this manner, with persistently good response. Each diuresis caused a fall in the serum chloride level, which would then rise with continuing administration of ammonium chloride.

Comment on Case 3.—This patient had a fairly characteristic course. His chloride level rose more rapidly when mercurial injections were not given than when he received mercurials and ammonium chloride. It is worthy of mention that mercurials may increase urinary chloride even when no diuresis occurs. Hence, continued administration without diuresis not only increases the danger of mercury poisoning but may aggravate the already existing low-chloride alkalosis.

This patient is typical of those who are "refractory" to mercurials with severe chloride depletion (85.5 mEq. per liter) but who have diuresis when moderate chloride reduction is present (92.3 mEq. per liter). The case again emphasizes the individuality of response and the fact that diuresis continues in some patients in spite of chloride levels below normal values.

Case 4.—A 49-year-old white man was admitted in frank congestive heart failure. He was known to have had hypertensive cardiovascular disease with bouts of decompensation for three years. Mercurial injections had recently become ineffective, in contrast to good diureses obtained formerly. Physical examination disclosed the findings of advanced heart failure, including hepatosplenomegaly and ascites.

In view of his poor response to the mercurial compound, hypochloremic alkalosis was suspected. The serum chloride was found to be 94.8 mEq. per liter. He was given ammonium chloride, 2 gm. four times daily. The mercurial injections were stopped. This dosage of ammonium chloride was continued for five days. On the sixth day his serum chloride was found to be 121.8 mEq. per liter. He was given 2 cc. of mercaptomerin on this and on the following day, resulting in a massive diuresis and a weight loss of 13 lb. (5.9 kg.) over a four-day period. The serum chloride level following the diuresis was 102.7 mEq. per liter. The patient improved markedly and was discharged fairly well compensated on the 12th hospital day.

Comment on Case 4.—Again a typical response was obtained by the simple expedient of withholding the injections of mercurials and replenishing chloride with ammonium chloride administration. This case is another example of striking decrease in serum chloride following mercurial injections. It is important that a sufficiently high chloride intake is maintained in this patient as long as mercurials are required. More frequent blood chemical determinations are also indicated if the patient is an abnormal "chloride loser" on mercurial diuresis.

GENERAL COMMENT

The mechanism of mercurial diuresis has been extensively investigated in recent years,[5a] and the evidence points to a renal site of action. In all likelihood mercury promotes diuresis by inhibiting reabsorption of sodium and/or chloride in the renal tubule.[6] Other electrolytes are affected, but less consistently and less strikingly. The marked increase in urinary chloride following administration of mercury, sometimes associated with lowering of serum chloride, was noted by Saxl and Heilig in 1920.[7] In 1925 Keith and Whelan observed a low serum chloride in several of their patients who were refractory to merbaphen (novasurol®).[8] They commented on the striking diuresis which resulted after mercurial injection when serum chloride was raised to a normal level with ammonium chloride. Keith again stressed the relationship of the serum chloride to refractoriness to mercurials in 1936.[9] However, no widespread practical application followed this observation. Ammonium chloride was widely employed on an empirical basis, but the reason for its frequent success was not clear.

Recently, interest in the relationship of hypochloremia to the refractory state has been rekindled by Schwartz.[3] He observed that restoration of depleted extra-

5a. Weston, R. E., and Escher, D. W.: An Analysis of the Unresponsiveness to Mercurial Diuretics Observed in Certain Patients with Severe Chronic Congestive Failure, J. Clin. Invest. **27**:561-562, 1948. Schroeder, H. A.: Studies on Congestive Circulatory Failure: IV. The Effect of Various Diuretics on the Excretion of Water and Chlorides, Circulation **4**:87-99, 1951. Stock, R. J.; Mudge, G. H., and Nurnberg, M. J.: Congestive Heart Failure: Variations in Electrolyte Metabolism with Salt Restriction and Mercurial Diuretics, ibid. **4**:54-69, 1951. Schroeder, H. A.: Use of Diuretic Agents, J. A. M. A. **147**:1109-1118, 1951. Newman, E. V.: Function of Kidney and Metabolic Changes in Cardiac Failure, Am. J. Med. **7**:490-496, 1949.

6. Schwartz and Wallace.[3c] Schmitz, H. L.: Studies on Action of Diuretics: Effect of Euphyllin and Salyrgan upon Glomerular Filtration and Tubular Reabsorption, J. Clin. Invest. **11**:1075-1097, 1932. Pitts, R. F., and Duggan, J. J.: Studies on Diuretics: The Relationship Between Glomerular Filtration Rate, Proximal Tubular Absorption of Sodium and Diuretic Efficacy of Mercurials, ibid. **29**:372-379, 1950. Pitts, R. F., and Sartorius, O. W.: Mechanism of Action and Therapeutic Use of Diuretics, Pharmacol. Rev. **2**:161-266, 1950.

7. Saxl and Heilig, cited by Crawford, J. H., and McIntosh, J. F.: Use of Novasurol in Edema Due to Heart Failure, J. Clin. Invest. **1**:333-358, 1925.

8. Keith, N. M., and Whelan, M.: Action of Ammonium Chloride and Organic Mercury Compounds, J. Clin. Invest. **3**:149-202, 1926.

9. Keith, N. M.: The Pharmacopeia and the Physician: Action and Use of Diuretics with Especial Reference to Mercurial Compounds, J. A. M. A. **107**:2047-2051, 1936.

cellular chloride stores was consistently accompanied with renewed response to the mercurial compound, and he also commented on the relative frequency of this situation.

The unfavorable influence of alkalosis on mercurial diuresis has been well demonstrated.[10] Administration of various alkalinizing salts will inhibit diuresis from mercurials. Conversely, administration of acidifying salts potentiates mercurial diuresis.[10a] The exact role of acidosis per se in enhancing mercurial diuresis is not clear. Recent studies suggest that the serum electrolyte pattern rather than the pH is the determining factor.[11]

The syndrome of hypochloremic alkalosis differs from the low-salt syndrome in several important respects. The carbon-dioxide-combining power is normal or elevated. The serum sodium usually remains normal. Azotemia is not ordinarily present. The patients will recover their response to mercurial diuretics following a high chloride intake without the addition of sodium. In contrast, those patients

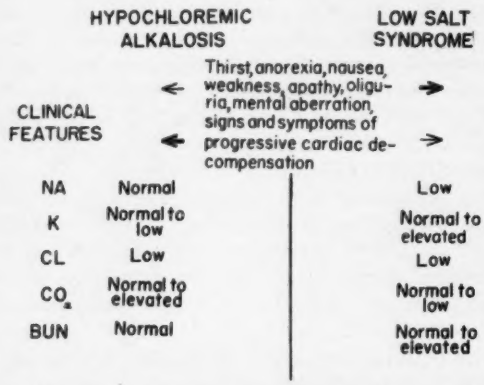

Fig. 4.—Essential clinical and laboratory features of hypochloremic alkalosis compared with those of low-salt syndrome. Progressive, refractory congestive failure is the predominating feature of hypochloremic alkalosis, whereas symptoms of dehydration and salt depletion characterize the low-salt syndrome. Overlapping of the symptomatology occurs as indicated by arrows. Values listed under hypochloremic alkalosis presume the absence of renal insufficiency.

with the low-salt syndrome tend to have a low carbon-dioxide-combining power, low serum sodium, and azotemia. The administration of hypertonic sodium chloride solution may be beneficial in these patients. In both situations the serum chloride is diminished (Fig. 4).

These syndromes often cannot be sharply distinguished on clinical grounds, as there are overlapping features. The diagnosis is, therefore, based on laboratory data.

10. Ethridge, C. B.; Myers, D. W., and Fulton, M. N.: Modifying Effect of Various Inorganic Salts on Diuretic Action of Salyrgan, Arch. Int. Med. **57**:714-728, 1936.

10a. Crutchfield, A. J., Jr., and Wood, J. E., Jr.: Role of Ammonium Chloride and Other Acid Producing Substances in Treatment of Congestive Failure, Virginia M. Month. **75**:381-385, 1948.

11. Hilton, J. G.: Potentiation of Diuretic Action of Mercuhydrin by Ammonium Chloride, J. Clin. Invest. **30**:1105-1110, 1951. Axelrod, D. R., and Pitts, R. F.: The Relationship of Plasma pH and Anion Pattern to Mercurial Diuresis, ibid. **31**:171-179, 1952.

When the question of electrolyte imbalance is raised, determination of serum chloride, carbon-dioxide-combining power, and blood urea nitrogen (or nonprotein nitrogen) will quickly orient one to the electrolyte situation. A serum sodium estimation is helpful, although not essential.

Once the diagnosis is established, use of mercurials is stopped. Ammonium chloride is administered in doses of 6 to 8 gm. by mouth daily. An occasional patient requires more. Ammonium chloride (1% solution) may be administered intravenously to those patients incapable of tolerating or absorbing the medicament orally. This solution should be given slowly, at a rate not exceeding 200 cc. an hour, so as to avoid toxic levels of ammonium ions in the blood.[12] Four to 8 gm. a day may be given in this manner.

Diluted hydrochloric acid U. S. P. may be used to supplement ammonium chloride in dosage of 5 cc. in a full glass of water four times daily, with the precautions of drinking this through a glass straw, followed by rinsing and brushing the teeth. This quantity of hydrochloric acid (i. e., 20 cc. of 10% solution) is equivalent in chloride content to approximately 3 gm. of ammonium chloride. Patients whose chloride requirement is not large but who are unable to tolerate ammonium chloride given orally may be maintained on the above dose of diluted hydrochloric acid. This amount of chloride apparently supplements dietary chloride sufficiently to restore response to mercurials in patients whose chloride loss has not been massive. This observation has been made by Schwartz [3a,c] and by one of us (W. P. H.).

With periodic serum chloride determinations, it is possible to follow the rising extracellular chloride level. When this value reaches a normal range, a mercurial is again injected. The response will usually be gratifying.

There seems to be a variance in the individual diuretic response to mercurial injections. Some patients have much greater chloride loss in their urine than others and are, therefore, more likely to develop a refractory state. Those not having such a marked drop in chloride with diuresis continue to respond well to frequent mercurial injections. The reason for this discrepancy in individual response is not well understood. The development of refractoriness to mercurials is, therefore, unpredictable; neither the type of patient who will become unresponsive nor the amount of mercurial that will cause this lack of response can be foretold. Theories to explain this difference in response have been advanced for many years and will not be repeated in this discussion.

The level of chloride at which patients may become refractory to mercurial diuresis also varies. Some fail to respond even though the chloride level is in the normal range (usually low normal), and apparently in some patients the elevation of a few milliequivalents causes renewed response. This suggests that there is an individual range, which is sometimes narrow but is of importance in certain cases.

The duration of intensive ammonium chloride therapy to elevate the serum chloride depends upon the degree of depletion. Usually several days to one week is sufficient, although longer periods may be required. It is important to stress that mercurial injections should not be given during this time in order to facilitate replenishing of the chloride stores.

12. Schwartz and Wallace,[3b] Karr, N. W., and Hendricks, E. L.: Toxicity of Intravenous Ammonium Compounds, Am. J. M. Sc. **218**:302-307, 1949.

Although ammonium chloride has been used as an adjuvant to mercurial therapy for years with considerable success, it has generally been used on an empirical rather than a rational basis. For this reason, some patients amenable to the technique described above have not benefited from ammonium chloride. In these patients chloride stores became depleted in spite of apparently adequate ammonium chloride administration because chloride output exceeded chloride intake over a period of time. In some patients this has resulted from too-frequent injections of mercurials or the use of excessive doses. In other patients dosage of ammonium chloride may not have been sufficient to compensate for a large loss of chloride in the urine. An occasional cause of failure has been poor absorption of ammonium chloride tablets in the gastrointestinal tract. These tablets at times have been found in the stools and occasionally have been seen in the lower bowel on a roentgenogram of the kidney, ureter, and bladder. Vomiting or diarrhea may be contributing factors.

It must be kept in mind when using mercurials that a certain level of tubular suppression exists, beyond which larger doses increase diuresis only slightly at much greater risk. A dose larger than 2 cc. is seldom indicated. In a susceptible person, even a small dose of a mercurial may induce a massive diuresis and refractoriness. It also appears to be poor policy to administer mercurials until no further weight loss occurs, the so-called dry weight, for this is possibly a state of dehydration of varying severity. The weight at which the patient appears best clinically is probably a better guide. Mercurials have been shown to be capable of dehydrating normal persons.[13]

It is worthy of emphasis that these cases represent a clinical and not a metabolic study and are presented to emphasize one phase of so-called refractory heart failure that is amenable to therapy. It is recognized that many facets are present in cases of congestive heart failure that are no longer responsive to treatment. The syndrome of hypochloremic alkalosis is only one of the facets, but its recognition may be lifesaving.

SUMMARY

The development of refractoriness to mercurial diuretics often presages an unmanageable downhill course in patients with congestive heart failure.

This refractory state at times is due to a disturbance of electrolyte balance. The disturbance may take several forms. The two most clearly defined types are the low-salt syndrome and the hypochloremic alkalosis syndrome. The latter syndrome is emphasized as a frequent but not well-publicized complication of the use of mercurial diuretics. The relative ease of diagnosis and treatment makes its consideration mandatory in every case of refractory heart failure.

Four illustrative cases are presented, and management of the syndrome is discussed.

Dr. Harold Jeghers and Dr. Laurence H. Kyle, of Georgetown University School of Medicine, gave helpful suggestions. Dr. Frank S. Horvath, Dr. Joseph J. McCarthy, Dr. Robert F. Owens, and Dr. William B. Walsh gave permission to include descriptions of their private patients in this report.

13. Lyons, R. H.; Jacobson, S. D., and Avery, N. L., Jr.: Change in Plasma Volume and Body Weight in Normal Subjects After Low Salt Diet, Ammonium Chloride and Mercupurin, Am. J. M. Sc. **211**:460-466, 1946.

CONTROLLED HYPOXEMIA TEST FOR CORONARY INSUFFICIENCY EMPLOYING THE MILLIKAN OXIMETER

A Study of Normal and Abnormal Responses

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THE DIAGNOSIS of coronary artery disease in the presence of a normal resting electrocardiogram has been aided by the use of tests designed to create a relative hypoxemia of the myocardium, the best known of which are the Master two-step test [1] and the anoxemia test of Levy.[2] The former method relies upon a standardized amount of exercise based on age, weight, and sex to create a greater demand for oxygen by the myocardium, whereas the anoxemia test decreases the amount of oxygen to the heart through the inhalation of a 10% oxygen mixture for a 20-minute period.

The electrocardiographic changes which occur with these procedures in the presence of coronary disease are reflected mainly by RS-T segment depressions

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1. (*a*) Master, A. M.; Friedman, R., and Dack, S.: The Electrocardiogram After Standard Exercise as a Functional Test of the Heart, Am. Heart J. **24**:777, 1942. (*b*) Master, A. M.: The Electrocardiogram and the "2-Step" Exercise, Am. J. M. Sc. **207**:435, 1944. (*c*) Unterman, D., and DeGraff, A. C.: The Effect of Exercise on the Electrocardiogram (Master "2-Step" Test) in the Diagnosis of Coronary Insufficiency, ibid. **215**:671, 1948. (*d*) Master, A. M.: The 2-Step Exercise Electrocardiogram: A Test for Coronary Insufficiency, Ann. Int. Med. **32**:842, 1950. (*e*) Chesky, K.; Pordy, L., and Master, A. M.: The "2-Step" Exercise Electrocardiogram: A Preliminary Follow-Up Study of 200 Cases, Bull. New York Acad. Med. **27**:383, 1951.

2. (*a*) Levy, R. L.; Bruenn, H. G., and Russell, N. G., Jr.: The Use of Electrocardiographic Changes Caused by Induced Anoxemia as a Test for Coronary Insufficiency, Am. J. M. Sc. **197**: 241, 1939. (*b*) Levy, R. L.; Patterson, J. E.; Clark, T. W., and Bruenn, H. G.: The "Anoxemia Test" as an Index of the Coronary Reserve: Serial Observations on 137 Patients with Their Application to Detection and Clinical Course of Coronary Insufficiency, J. A. M. A. **117**:2113, 1941. (*c*) Levy, R. L.; Williams, N. E.; Bruenn, H. G., and Carr, H. A.: The "Anoxemia Test" in the Diagnosis of Coronary Insufficiency, Am. Heart J. **21**:634, 1941. (*d*) Björck, G.: Anoxemia and Exercise Test in the Diagnosis of Coronary Disease, ibid. **32**:689, 1946. (*e*) Burchell, H. B.; Pruitt, R. D., and Barnes, A. R.: The Stress and the Electrocardiogram in the Induced Hypoxemia Test for Coronary Insufficiency, ibid. **36**:373, 1948. (*f*) Nylin, G.; DeFazio, V., and Marsico, F.: The Hypoxemia Test: An Analysis of 1,130 Tests, Cardiologia **17**:191, 1950. (*g*) Mathers, J. A., and Levy, R. L.: The Prognostic Significance of the Anoxemia Test in Coronary Heart Disease, Am. Heart J. **43**:546, 1952.

and/or T wave inversions in one or several leads. These alterations are similar in both tests and are thought to be due to factors common to the effects of exercise or hypoxia upon the myocardium.[3]

The degree of myocardial hypoxemia has been stressed by several investigators as an important factor in the production of changes in the electrocardiogram.[4] Since many normal hearts after heavy exercise or pronounced hypoxemia will show electrocardiographic changes indistinguishable from coronary insufficiency patterns,[5] it is desirable to determine the amount of activity or hypoxemia normal persons can tolerate without displaying significant electrocardiographic aberrations. Abnormalities seen in a significant percentage of normal young adult controls who are statistically reasonably free from coronary artery disease[6] should be excluded from criteria of a positive test result.

Single and double two-step tests were recently performed on 200 normal persons between the ages of 17 and 57 by Master's group.[7] Ninety-nine of these subjects were under 30 years of age. Based upon the single criterion of RS-T segment depression greater than 0.5 mm., 9% of these young adults had positive tests. Of the entire group, 11.5% had positive tests, consisting of abnormal RS-T segment depression in 13, flat T waves in 4, and cardiac arrhythmias in at least 6. Master's criteria for a positive test are as follows:

1. Depression of the RS-T segment below the isoelectric level (P-Q level) of more than 0.5 mm. in Leads I, II, or IV.

2. Change from an upright T wave to an isoelectric or inverted T wave (or change of T wave in the opposite direction).

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3. Baum, W. S.; Malmo, R. B., and Sievers, R. F.: A Comparative Study of the Effects of Exercise and Anoxia upon the Human Electrocardiogram, J. Aviation Med. **16**:422, 1945.

4. (*a*) Rothschild, M. A., and Kissin, M.: Induced General Anoxemia Causing S-T Deviation in the Electrocardiogram, Am. Heart J. **8**:745, 1933. (*b*) Dripps, R. D., and Comroe, J. H., Jr.: The Effect of the Inhalation of High and Low Oxygen Concentrations on Respiration, Pulse Rate, Ballistocardiogram and Arterial Oxygen Saturation (Oximeter) of Normal Individuals, Am. J. Physiol. **149**:277, 1947. (*c*) Malmström, G.: The Cardiological Anoxemia Test with Special Reference to Its Standardization: Clinical and Physiological Study, Acta med. scandinav., Supp. 195, 1947, p. 1. (*d*) Penneys, R., and Thomas, C. B.: Oximeter Control of Arterial Oxygen Saturation in Anoxemia Studies, Bull. Johns Hopkins Hosp. **82**:470, 1948; (*e*) The Relationship Between the Arterial Oxygen Saturation and the Cardiovascular Response to Induced Anoxemia in Normal Young Adults, Circulation **1**:415, 1950.

5. (*a*) Kountz, W. B., and Gruber, C. M.: The Electrocardiographic Changes in Anoxemia, Proc. Soc. Exper. Biol. and Med. **27**:170, 1929. (*b*) Hoogerwerf, S.: Elektrokardiographische Untersuchungen der Amsterdamer Olympiakämpfer, Arbeitsphysiologie **2**:61, 1929-1930. (*c*) Kountz, W. B., and Hammouda, M.: Effect of Asphyxia and of Anoxemia on the Electrocardiogram, Am. Heart J. **8**:259, 1932. (*d*) Riseman, J. E. F.; Waller, J. V., and Brown, M. G.: The Electrocardiogram During Attacks of Angina Pectoris: Its Characteristics and Diagnostic Significance, ibid. **19**:683, 1940. (*e*) Burnett, C. T.; Nims, M. G., and Josephson, C. J.: The Induced Anoxemia Test: Study by Age Groups, ibid. **23**:306, 1942.

6. Wartman, W. B.: The Incidence and Severity of Arteriosclerosis in the Organs from 500 Autopsies, Am. J. M. Sc. **186**:27, 1933; Factors Concerned in Narrowing or Occlusion of Coronary Vessels, Blood, Heart, and Circulation, Publication No. 13, Washington, D. C., American Association for the Advancement of Science, 1940, p. 122.

7. Scherlis, L.; Sandberg, A. A.; Wener, J.; Dvorkin, J., and Master, A. M.: The Effects of the Single and Double "2-Step" Exercise Tests upon the Electrocardiograms of 200 Normal Persons, J. Mt. Sinai Hosp. **17**:242, 1950.

3. The presence of arrhythmia immediately after exercise which was not present before exercise and which reverts to normal.

Other investigators have considered exercise tests not positive unless the RS-T segment depression is 0.75 mm. or more.[8]

In Levy's original investigation to determine the limits of normal response to anoxemia, 66 controls of 20 to 68 years of age were studied.[2a] The blood oxygen levels were stated to be variable, although no actual levels were reported. He concluded that there was no direct correlation between the degree or length of hypoxemia and the magnitude of change in the electrocardiogram. RS-T segment depressions of less than 1 mm. were considered normal. Twenty-two of these subjects showed partial or complete T wave reversal in Lead II. In a subsequent study on clinically normal controls varying in age from 21 to 78 years, he noted:

> In a number of our subjects who were regarded as normal, and especially in the younger age groups, changes in the electrocardiogram were observed during the first test which were not present, or were of lesser degree, when the test was repeated. . . .
>
> The criteria for an abnormal response were made sufficiently rigid so that normal subjects who showed such variation were excluded from the group with coronary insufficiency.[2c]

A later investigation of 136 normal subjects between 21 and 73 years of age showed 1.5-mm. RS-T depression in 1 subject and 1-mm. RS-T depressions in 21.[9] Reversal of T waves was seen in Lead I in 1.5%, in Lead II in 5.1%, and in Lead IVF in 5.9%. Based on these studies, Levy established the following criteria of an abnormal response:

1. The sum of the RS-T deviations in Leads I, II, III, and IVF should be greater by 3 mm. or more than that in the control tracing.

2. There should be partial or complete reversal of the direction of the T wave in Lead I, accompanied with an RS-T deviation of 1 mm. or more in this lead.

3. Complete reversal in the direction of the T wave should be present in Lead IVF, regardless of any associated RS-T deviation in this lead.

In none of the reports referred to above, however, was the arterial oxygen concentration correlated with electrocardiographic changes. It has been shown that breathing 10 or 12% oxygen mixtures will result in wide fluctuations of the arterial oxygen saturation varying from 50.1 to 91% in different persons.[10] This variation

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8. (*a*) Unterman and DeGraff.[1c] (*b*) Twiss, A., and Sokolow, M.: Angina Pectoris: Significant Electrocardiographic Changes Following Exercise, Am. Heart J. **23**:498, 1942. (*c*) Feil, H., and Pritchard, W. H.: The Electrocardiogram of Anginal Attacks Induced by Exercise, Mod. Concepts Cardiovas. Dis. **15**:4, 1946. (*d*) Grossman, M.; Weinstein, W. W., and Katz, L. N.: The Use of the Exercise Test in the Diagnosis of Coronary Insufficiency, Ann. Int. Med. **30**:387, 1949.

9. Patterson, J. E.; Clark, T. W., and Levy, R. L.: A Comparison of Electrocardiographic Changes Observed During the "Anoxemia Test" on Normal Persons and on Patients with Coronary Sclerosis, Am. Heart J. **23**:837, 1942.

10. (*a*) Burchell and others.[2e] (*b*) Dripps and Comroe.[4b] (*c*) Malmström.[4c] (*d*) Levy, R. L.: Barach, A. L., and Bruenn, H. G.: Effects of Induced Oxygen Want in Patients with Cardiac Pain, Am. Heart J. **15**:187, 1938. (*e*) Houston, C. S.: The Effect of Pulmonary Ventilation on Anoxemia, Am. J. Physiol. **146**:613, 1946. (*f*) Mathers, J. A. L., and Levy, R. L.: Correlation of the Oxygen Saturation of the Blood and Changes in the Electrocardiogram, Blood Pressure, and Heart Rate During the Anoxemia Test, Circulation **1**:426, 1950.

is apparently a function of the rate and depth of pulmonary ventilation. It has also been demonstrated that, with the use of Levy's criteria, breathing a mixture containing the equivalent of 8.29% oxygen will result in a 19% incidence of abnormal electrocardiograms in normal controls.[5e]

In the study of patients with coronary insufficiency, it has been stated that the level of arterial oxygen saturation is not of critical importance in eliciting a positive reaction to the anoxemia test.[11] As evidence for this, Burchell and his group noted that patients having clinical coronary artery disease with negative reactions to the test had arterial oxygen saturations similar to those of persons with positive reactions.[2e] In their series, there were nine patients with anginal pain who had negative or equivocal tests and whose arterial oxygen saturation varied from 74 to 85%. Ten others with the same syndrome and positive tests had arterial oxygen levels of 76 to 83% saturation.

Similarly, Mathers and Levy performed 109 anoxemia tests on 97 persons, 45 of whom had coronary sclerosis, and observed the blood oxygen saturations with the use of a Millikan oximeter.[10f] Sixteen persons had oxygen saturations above 80% on their first test, and three of these showed positive records. A total of 21 abnormal responses was obtained at arterial oxygen saturations varying from 64 to 85%. They concluded that no optimal range of arterial oxygen desaturation could be defined which caused electrocardiographic changes characteristic of a positive test.

In order to study the effects of anoxemia on the electrocardiogram, Penneys and Thomas employed a method of controlling the blood oxygen concentration by the use of the Millikan oximeter and variable mixtures of oxygen and nitrogen supplied through an anesthesia machine.[4e] Seventy-six normal adults, only one of whom was more than 30 years old, were studied at various levels of desaturation. It was shown that the electrocardiographic changes progressively increased as the oxygen content of the blood fell. Thus, at 80% saturation, 6% of the persons studied showed RS-T segment deviations of 1 mm. At 75% saturation, 22% showed this change, and at 70% the figure had increased to 43% of those observed. A similar progressive decrease in the amplitude of the T waves was noted. Of 28 persons studied at 70% saturation, none showed an RS-T segment deviation greater than 1 mm. Diphasic T waves in Lead CF4 occurred twice at 75%, and an inverted T wave in Lead II was seen once at 70%. Prolongation of hypoxemia at any given level caused no progressive changes in the electrocardiogram.

Storch and Master compared the results of the two-step test with those of the anoxemia test in 23 subjects and, after exercise, found 11 positive tests by their criteria and 5 positive anoxemia tests by Levy's criteria.[12] The arterial oxygen level was not correlated in their study of the anoxemia test.

Because of these different viewpoints concerning the importance of the blood oxygen level and the apparent lack of correlation between the anoxemia and two-step tests, we became interested in studying further the electrocardiographic changes in normal subjects and in subjects with coronary insufficiency in whom the oxygen content of the blood could be controlled. In addition, we wished to compare our

11. Burchell and others.[2e] Mathers and Levy.[2g] Mathers and Levy.[10f]

12. Storch, S., and Master, A. M.: RS-T Segment, T Wave and Heart Rate After 2-Step and 10% Anoxemia Tests, J. A. M. A. **146**:1011, 1951.

results with this method with the electrocardiogram after exercise and, finally, to evaluate the two tests from the standpoint of sensitivity, simplicity, and incidence of unpleasant reactions.[13]

METHOD

Hypoxemia was induced essentially by the method described by Penneys and Thomas.[14] A Foregger anesthesia machine was used to deliver variable mixtures of oxygen and nitrogen via an O. E. M. mask with an escape valve to prevent rebreathing. The arterial oxygen content was measured with the Millikan automatically compensated oximeter.[15]

With the subject in a supine position and breathing 100% oxygen, a 12-lead control electrocardiogram utilizing a direct-writing instrument was taken and the oximeter calibrated at 97%. The oxygen content of the inspired gas was then reduced to whatever rate of flow was necessary to cause the arterial oxygen content to fall about 5% every few minutes.

Segments of Leads II and V_4 or V_5 were taken with every 5% decrease in the arterial oxygen saturation. When it had reached 70% saturation, the oxygen flow was increased to maintain the patient at this level, and a second electrocardiogram was obtained. The longest time any subject remained at this level of desaturation was 20 minutes. If an abnormal electrocardiogram developed before 70% saturation was reached, the subject was maintained at the level at which the change occurred, a tracing was obtained, and 100% oxygen administered.

The two-step test was performed in accordance with Master's specifications.[1d]

Both tests were done on the same day after an hour's rest in several subjects showing normal responses. However, no person with an abnormal reaction was subjected to the second test in less than 24 hours.

Hypoxemia tests were performed on 55 subjects, all but 5 of whom were carried to 70% arterial oxygen saturation. Twenty of these subjects were healthy men ranging in age from 23 to 38 years and serving as normal controls. Of the 35 other subjects, 4 were in their fourth decade, and the remaining 31 varied from 40 to 64 years of age.

Three patients in the group more than 40 years of age had anginal pain (Cases 25, 29, and 32), and one had a history of ancient posterior myocardial infarction (Case 30). All the rest were free from clinical stigmata warranting a diagnosis of coronary artery disease. Except for a small Q wave in Leads III and AVF in Case 30, there were no abnormal QRS complexes, RS-T segment deviations, or T wave inversions in this group. There were, however, numerous instances of upright T waves of low amplitude.

Two-step tests were performed on 37 subjects exclusive of the control group.

RS-T segment measurements were made, employing the P-Q segment as the isoelectric line.

RESULTS

Normal Electrocardiographic Response to Hypoxemia.—All 20 control subjects attained 70% arterial oxygen saturation, and 8 were maintained at this level for 20 minutes. The latter practice was discontinued when it was observed that no greater response could be expected in the electrocardiogram at the end of 20 minutes than was seen in tracings obtained immediately after the 70% level was reached.

At variance with the results of Penneys' study, no subject showed an RS-T deviation of 1 mm. The greatest response noted was a 0.5-mm. depression of RS-T

13. Chesky and others.[1e] Footnote 2*a* to *d* and *f*. Malmström.[4c] Burnett and others.[8e] Footnote 8*c* and *d*. Levy and others.[10d] Storch and Master.[12] Alexander, S. F.; Wittenberg, S. J.; Brown, E. T., and Koffler, A.: Induced Cardiac Anoxemia and Coronary Artery Disease, New York J. Med. **50**:535, 1950.

14. Penneys and Thomas.[4d] Penneys, R.; Thomas, C. B., and McLean, R. L.: A Constant Degree of Anoxemia Obtained by the Administration of a Gas of Variable Oxygen Concentration, Bull. Johns Hopkins Hosp. **84**:430, 1949.

15. Millikan, G. A.: The Oximeter: An Instrument for Measuring Continuously the Oxygen Saturation of Arterial Blood in Man, Rev. Scient. Instruments **13**:434, 1942.

TABLE 1.—*Comparison of RS-T and T Wave Changes Before and After Hypoxemia and Exercise*

Maximum RS-T Deviation in Mm. After Hypoxemia and 2-Step Tests*

Case No.	Age	Sex	Arterial O_2 Saturation, %	2-Step	Lead I		Lead II		Lead III		Lead V_4		Lead V_5		Lead V_6	
					Hypoxia	2-Step	Hypoxia	2-Step	Hypoxia	2-Step	Hypoxia	2-Step	Hypoxia	2-Step	Hypoxia	2-Step
21	64	M	85	Sngl	0.25	0.50	0.25	0.50	0.00	0.25	1.25	1.25	1.00	0.75	0.25	0.75
22	47	M	70	Dbl	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
23	52	F	70	Dbl	0.50	0.25	0.75	0.75	0.25	0.25	1.50	1.50	1.00	1.00	0.50	0.50
24	50	F	70	Dbl	0.50	0.00	0.00	0 25	0.00	0.00	0.50	0.00	1.00	0.25	0.50	0.00
25	61	F	85	Sngl	1.00	0.50	0.00	0.50	+1.00	0.00	2.50	2.50	3.00	3.00	....	2.50
26	58	M	70	Dbl	0.50	0.25	0.75	0.50	0.00	0.00	1.00	0.50	1.50	1.50	0.75	0.50
27	53	F	70	Dbl	0.50	0.00	0.50	0.00	0.00	0.00	1.50	0.50	0.50	0.50	0.50	0.50
28	56	M	70	Sngl	1.25	1.00	1.50	1.50	0.00	0.00	4.00	2.50	3.00	1.50	2.00	2.00
29	59	F	70	Sngl†	0.75	0.00	0.75	0.00	0.00	0.00	0.50	0.50	0.50	0.50	1.00	0.50
30	42	M	70	Sngl	0.50	0.00	+0.50	0.00	+0.75	0.00	2.00	1.00	2.25	2.00	0.50	1.25
31	61	F	74	Sngl	0.50	0.25	0.25	0.75	0.00	0.00	1.00	1.00	1.50	1.50	0.75	2.00

Maximum Changes in Amplitude of T Waves in Mm. Before and After Hypoxemia and 2-Step Tests

Case No.	Lead I				Lead II				Lead V_4				Lead V_5				Lead V_6			
	Hypoxemia Control	2-Step Control	Hypoxemia Test	2-Step Test	Hypoxemia Control	2-Step Control	Hypoxemia Test	2-Step Test	Hypoxemia Control	2-Step Control	Hypoxemia Test	2-Step Test	Hypoxemia Control	2-Step Control	Hypoxemia Test	2-Step Test	Hypoxemia Control	2-Step Control	Hypoxemia Test	2-Step Test
21	0.75	0.75	0.50	0.50	0.50	0.50	0.25	0.25	2.00	1.50	0.50	1.50	1.25	1.00	0.50	1.00	1.00	0.75	0.25	0.75
22	0.25	0.25	0.00	0.00	1.00	0.50	0.00	0.00	2.00	1.50	1.50	1.50	1.00	0.50	0.25	0.25	0.75	0.25	0.00	0.00
23	1.00	2.50	0.75	1.00	2.00	2.00	0.75	0.75	3.50	2.00	0.75	1.25	3.50	2.25	1.25	1.00	3.00	2.00	0.75	1.00
24	0.50	1.00	−0.50	1.00	0.75	1.00	0.25	0.75	2.50	1.50	−1.75	1.25	2.00	1.25	−2.25	1.50	1.75	1.00	−1.00	1.25
25	1.50	1.75	0.50	0.50	1 00	0.75	0.00	0.25	4.00	3.00	4.00	6.50	2.50	3.00	1.75	5.00	1.50	2.00	....	2.50
26	1.00	2.75	0.75	2.75	1.50	3.00	1.00	3.50	3.50	2.50	2.00	5.25	3.00	3.00	1.50	5.00	3.00	2.50	1.25	3.50
27	1.50	2.50	−0.50	0.50	2.00	3.50	0.25	1.50	2.00	3.00	−4.00	1.00	1.75	3.25	−1.50	0.75	1.50	3.00	−1.00	0.25
28	0.75	0.50	0.00	0.50	1.25	0.75	0.25	0.50	3.00	1.00	1.50	1.25	2.25	1.50	1.00	0.50	2.00	0.75	0.50	0.25
29	0.75	0.50	0.00	0.25	1.75	1.00	0.00	0.50	1.00	1.25	0.00	−1.00	1.25	0.50	−1.00	0.25	1.00	0.50	−1.00	0.25
30	1.00	3.25	0.25	1.25	0.25	2.00	1.75	1.75	8.50	9.00	0.25	6.50	7.50	7.50	0.25	5.00	3.50	3.75	0.50	2.75
31	0.50	0.75	0.00	0.25	1.50	0.75	0.50	1.00	1.50	3.50	−2.00	5.00	0.75	2.00	−3.50	1.75	0.25	1.25	−1.50	1.50

* Figures represent depressions unless preceded by plus sign.
† Five-minute record. Tracing immediately after exercise was normal.

in Lead V_5 on one occasion. Except in Lead III, no flat or inverted T waves occurred, although considerable diminution of T wave amplitude developed in almost every case.

In 18 persons over the age of 40, the electrocardiographic response at 70% arterial oxygen saturation was indistinguishable from that described above, although the incidence of 0.5-mm. RS-T depressions was increased. The oldest subject in this group was 58 years of age. Seven were maintained at 70% arterial oxygen saturation for 20 minutes.

Abnormal Responses to Hypoxemia and the Two-Step Test.—Table 1 lists the RS-T and T wave changes observed in 11 patients showing positive reactions to either the two-step or the anoxemia test according to the appropriate criteria.[16]

It is interesting to note in Case 22 that the isoelectric T wave in Lead V_6 which renders the test positive by Master's criteria also occurred with the hypoxemia test. In Case 26 the hypoxemia test is negative by Levy's criteria, but the actual RS-T segment depression in each lead induced by hypoxemia is as great as or greater than

TABLE 2.—*Positive Tests*

Case No.	Master Criteria, 2-Step	Levy Criteria, Hypoxemia	Modified Criteria	
			2-Step	Hypoxemia
21	+	0	+	+
22	+	0	0	0
23	+	+	+	+
24	0	+	0	+
25	+	+	+	+
26	+	0	+	+
27	+*	+	0	+
28	+	+	+	+
29	+	+	+	+
30	+	+	+	+
31	+	+	+	+
	10	8	8	10

* 0.75-mm. RS-T depression in lead V_5.

that following exercise. In Case 21 the reaction is also negative by the Levy criteria, although greater depression of the RS-T segment would almost certainly have occurred with further lowering of the oxygen level. We discontinued the test at 85% saturation because at the time this patient was studied we believed an RS-T depression greater than 1 mm. to be an abnormal response to hypoxemia.

It is to be noted that six of the eight positive hypoxemia tests would have been negative by Levy's criteria if the arterial oxygen saturation had not reached 70%.

The last two columns in Table 2 compare the tests employing as positive criteria T wave inversion in any of the precordial leads or RS-T segment depression greater than 1 mm. in any lead. These standards were devised in deference to Penneys' frequent finding of 1-mm. RS-T segment depressions, Levy's observation of frequent T wave inversions in Lead II, and Master's 9% incidence of false-positive reactions in young adults.

16. One patient with an abnormal response to hypoxemia and a normal double two-step test is excluded because the arterial oxygen level at the time of the reaction is unknown (Case 46, see below). A second patient with tobacco angina had a positive double two-step and negative hypoxemia test at 74% oxygen saturation (Case 32). This record was also excluded because it was learned he had smoked prior to the exercise test but not before the hypoxemia test.

Reactions.—One 56-year-old psychoneurotic man complained of dizziness and faintness which prevented completion of the double two-step test, although he withstood 20 minutes of hypoxemia. His response to both tests was normal. No other reactions were noted during exercise tests.

About one-half of all patients complained of mild headaches or lightheadedness during the hypoxemia test, and troublesome hyperventilation occurred in two subjects with normal electrocardiographic responses. Chest pain occurred in two persons; both had positive tests (Cases 25 and 31).

The only disturbing reaction to hypoxemia occurred in a 55-year-old white man who had mild hypertension and emphysema (Case 46). The electrocardiogram was normal at 80% saturation. Fall in oxygen content apparently ceased at 78%, and the oxygen supply to the patient was further decreased. He became pale but stated that he felt well. Immediately thereafter, the oximeter recorded a saturation of 85%.[17] Before he could be questioned again, a brief clonic convulsion occurred and respiration ceased. One hundred per cent oxygen was promptly given, together with artificial respiration. An electrocardiogram taken during apnea showed an idioventricular rhythm with a rate of 40. Spontaneous respiration returned in about one and a half minutes, and recovery was complete. The patient had no complaints either before or after this episode.

COMMENT

We believe the single most important factor affecting the sensitivity and accuracy of the hypoxemia test to be the degree of the arterial oxygen desaturation It should be apparent that the myocardial reserve of any two patients with coronary sclerosis is not necessarily the same. Because one of these two patients has an abnormal response at 85% oxygen saturation and the other has a normal response at this level, one cannot presume that the oxygen content is not decisive in developing positive tests. The possibility exists that the other subject would display a positive record somewhere between 85 and 70%. On the basis of our findings, it is thought that anyone who has a normal test record at more than 70% arterial oxygen saturation is a potential candidate for a false-negative hypoxemia test. Needless to say, false-negative reactions will occur even at the recommended level, but their incidence should be reduced.

Levy wisely made his criteria rigid enough to exclude practically all false-positive reactions due to arterial oxygen desaturations below 70%. However, on the basis of Penneys' study of normal subjects and our own series, it is probable that the normal electrocardiographic response to hypoxemia, as outlined in this paper, is considerably less than is included in Levy's allowances for normal. Here again, the possibility exists for false-negative tests to occur.

Although the average arterial oxygen level with the Levy test will approximate a 70% saturation, this approximation is of no value in individual tests, for the arterial oxygen content is unknown and may vary considerably above or below this figure.

Utilizing a controlled decrease in oxygen content and frequent electrocardiograms, one can detect abnormal responses promptly. This method appears safer than waiting 10 minutes before obtaining a record. During such a period pronounced electrocardiographic changes may have developed and been allowed to persist at ever-decreasing blood oxygen levels.

17. We presume that a fall in blood pressure decreased the circulation to the ear, rendering it more transparent and thereby allowing more light to reach the photoelectric cell of the oximeter.

No comparison of the frequency of positive responses following exercise and controlled hypoxemia is justified from this study, although we feel that a larger series of cases would show little difference between the tests. We were not impressed with the necessity for two sets of criteria to delineate positive responses which are qualitatively similar although initiated by different stimuli. The criteria mentioned earlier should tend to eliminate false-positive reactions following the two-step test and false-negative reactions with the controlled hypoxemia test.

From the standpoint of freedom from reactions and simplicity, the two-step test is superior and supplies valuable information to the physician in the investigation of patients with suspected coronary artery disease. The controlled hypoxemia test as described in this paper requires expensive equipment and is not without danger. It is more easily quantitated, however, and is sensitive to small fluctuations in the arterial oxygen content. These factors suggest it to be the procedure of choice for cardiovascular research when a stress test is required.

SUMMARY AND CONCLUSIONS

Fifty patients were studied during induced hypoxemia of 70% arterial oxygen saturation.

In normal controls, no RS-T segment deviations greater than 0.5 mm. occurred. No flat or inverted T waves were noted.

The abnormal responses following exercise and hypoxemia are compared.

The importance of the arterial oxygen level in eliciting positive hypoxemia tests is stressed.

One severe reaction to hypoxemia is reported.

It is suggested that present criteria employed in both tests be modified.

The controlled hypoxemia test is an expensive and complicated procedure. The two-step test is more easily incorporated into clinical studies.

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RESIDUAL EFFECTS OF RICKETTSIAL DISEASE ON THE CENTRAL NERVOUS SYSTEM

Results of Neurologic Examinations and Electroencephalograms Following Rocky Mountain Spotted Fever

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ROCKY Mountain spotted fever is one of the severest of all infectious diseases. Essentially it is a specific generalized intracellular infection of small peripheral blood vessels. In untreated patients the rickettsias circulate in the blood during the first week and part of the second week after infection. The organisms first invade the nuclei of capillary endothelial cells, where they multiply in great numbers and destroy the cells. From there the lesion extends centripetally along the intima into slightly larger vessels (the arterioles), where smooth muscle cells of the media are also invaded and destroyed.[1] This destruction of muscle cells is a distinctive feature of Rocky Mountain spotted fever (Fig. 1). With the death of cells necrosis occurs in the intima and media of the vessels, resulting in thrombosis and extravasation of blood. As a result of the thrombosis, microinfarcts are formed, chiefly in the skin, subcutaneous tissues, and central nervous system (Fig. 2). As the disease progresses, perivascular accumulations of mononuclear macrophages develop and the vascular lesions assume a proliferative or granulomatous character (Fig. 3). In the central nervous system areas of demyelination may be found adjacent to or removed from the vascular lesions. Pathologic examination reveals greater damage to the brain in spotted fever than in any other rickettsial disease.

The acute phase of the infection is most frequently ushered in by headache, which develops before the rash appears. As the disease progresses, mental confusion, dulling of the senses, restlessness, or lethargy progressing to coma may be noted.[2] Muscular twitching, purposeless movements, fibrillary tremors, and abnormal neurologic signs, such as ankle clonus or a positive Babinski reaction, may occur. Indeed the picture often is one of encephalitis.

This study was supported in part by a grant from the John and Mary R. Markle Foundation.

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1. Lillie, R. D.: Pathology of Rocky Mountain Spotted Fever, Bulletin 177, National Institute of Health, Federal Security Agency, United States Public Health Service, 1941. Wolbach, S. B.: The Pathology of the Rickettsial Diseases of Man, in the Rickettsial Diseases of Man, Washington, D. C., American Association for the Advancement of Science, 1948, pp. 118-125.

2. Harrell, G. T., Jr.: Rocky Mountain Spotted Fever, Medicine **28**:333-370 (Dec.) 1949.

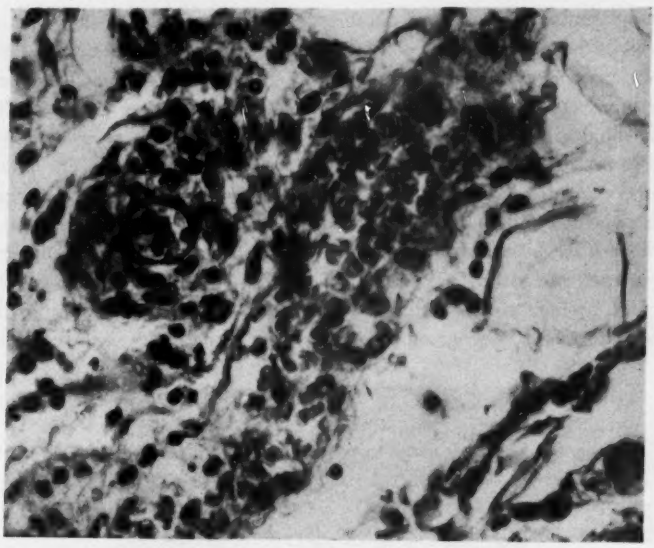

Fig. 1.—Arteriole from subcutaneous tissue of 7-year-old girl who died of rickettsial spotted fever on 11th day of rash. Necrosis of endothelium and muscle cells of wall is accompanied with perivascular inflammatory reaction.

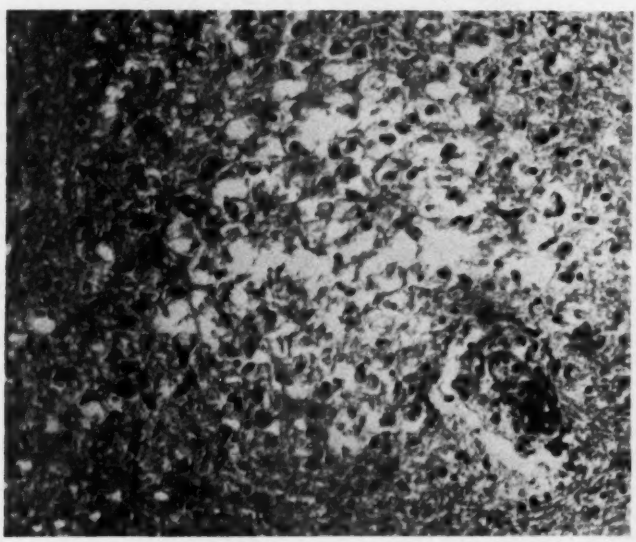

Fig. 2.—Microinfarct in brain of 4-year-old girl who died on 12th day of rash. Wall of arteriole in lower right portion of field is necrotic; lumen is partially occluded by thrombus. Cells of nerve tissue in center of field are disappearing.

During the acute phase, the cerebrospinal fluid frequently shows alterations which reflect the clinical evidence of encephalitis, although the cellular and chemical changes cannot be correlated closely with objective neurologic manifestations.[3] In many instances the pressure is normal; in other cases pressures as high as 300 mm. of spinal fluid are recorded. Almost every lumbar puncture reveals 1 to 20 red blood cells per cubic millimeter on taps performed without trauma. The white blood cells, predominantly small mononuclear forms, are often increased, numbering 10 to 22 cells per cubic millimeter. Elevations in globulin, as measured by the Pandy reaction, are rare. Total spinal fluid proteins are occasionally increased, sometimes reaching levels of 265 mg. per 100 cc. The low levels of globulin suggest that the spinal fluid protein is composed chiefly of the smaller albumin molecule and may indicate that early in the course of the disease the permeability of the cerebral capil-

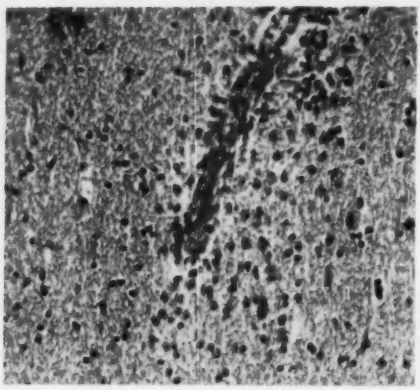

Fig. 3.—Granuloma from different portion of brain of 4-year-old girl. Wall of arteriole is damaged, and brain cells immediately adjacent are disappearing. Phagocytic cells are infiltrating area.

laries to proteins is not markedly increased. The spinal fluid sugar and chlorides are usually within the normal range.

The extent of damage resulting from the lesions in the skin and subcutaneous tissues can be easily followed by simple inspection, and the completeness of repair can be readily evaluated. In the central nervous system, however, it is difficult to estimate the extent of the acute lesions and the degree of residual damage, since the involved areas are so small and are diffusely scattered. Neither the clinical findings nor studies on the spinal fluid during the acute phase of the illness make it possible to predict which patients will be left with permanent damage to the nervous system. After convalescence, residual brain damage may be manifested clinically by loss of memory, pronounced mental retardation, behavior disorders, or even

3. Kelsey, W. M., and Harrell, G. T.: Management of Tick Typhus (Rocky Mountain Spotted Fever) in Children, J. A. M. A. **137**:1356-1361 (Aug. 14) 1948.

convulsions.[4] The observation of seizures led us to undertake the present study in an effort to determine the frequency and character of the sequelae in the central nervous system.

OUTLINE OF THE STUDY

Fifty-six patients of all ages have been successfully treated at the North Carolina Baptist Hospital for rickettsial spotted fever in the past eight years. Nineteen of these patients could not be traced, but the remaining 37 were reexamined one to eight years after the acute phase of the illness. At this time the neurologic aspects of the history and physical examination were emphasized, and an electroencephalogram was made. In some patients with clinical evidence of cerebral damage psychologic testing, including evaluation of the mental ability, was performed.

The data were studied in regard to the presence of positive neurologic findings during the febrile phase, the clinical severity of the illness, the duration of fever, the type of therapy, the history of neurologic symptoms after recovery, objective abnormalities in the neurologic reexamination, and changes in the electroencephalogram.

RESULTS

In 15 of the 37 patients with Rocky Mountain spotted fever neurologic abnormalities had been evident on physical examination during the acute illness. The disturbances observed included positive Babinski, Brudzinski, or Kernig signs, athetosis, spasticity, fasciculations, tremor, hyperreflexia, hyperesthesia, alterations in pupillary size or reflex, and nystagmus. When these patients were reexamined, three showed residual neurologic signs and two gave a history of having convulsions.

At the time of reevaluation 14 of the 37 patients complained of symptoms related to the central nervous system. These symptoms included headache, nervousness, backache similar to that present during the acute illness, emotional lability, lack of emotional control, overactivity, memory loss, choking, "shaking in the sleep," convulsions, dizziness, staggering, weakness, and inability to enjoy life.

Six patients showed abnormal neurologic signs; three of these had had neurologic abnormalities during the acute illness as mentioned above, while in the other three no objective changes had been noted during the febrile phase. These residual signs included impairment of fine motions, hypotonia, hyperreflexia, inequality of tendon reflexes, positive Babinski sign, ankle clonus, disturbances in gait, mental retardation, disturbances in behavior, and asymmetry or weakness of the muscles.

It should be noted, however, that not all the areas of muscular atrophy or asymmetry are necessarily indicative of damage to the central nervous sysetm. Small residual lesions may result from damage to peripheral nerves. Localized gangrene is known to follow destruction of the blood supply in the skin and subcutaneous tissues; repair of the tissues with scarring may result in small local areas of asymmetry.

Electroencephalographic tracings were clearly abnormal in 12 patients (Figs. 4 and 5). The abnormalities noted in the records were as follows: focal spike, spike and slow wave (eight cases), generalized beta waves (two cases), focal slow wave (one case), generalized four- to six-per-second activity (one case), and slow

4. (*a*) Harrell, G. T.; Masland, R. L., and Rosenblum, M. J.: Central Nervous System Sequelae as Shown by Post-Recovery Electroencephalograms in Rickettsial Spotted Fever, abstracted, J. Clin. Invest. **30**:645 (June) 1951. (*b*) Thomas, M. H., and Berlin, L.: Neurologic Sequelae of Rocky Mountain Spotted Fever, Arch. Neurol. & Psychiat. **60**:574-583 (Dec.) 1948.

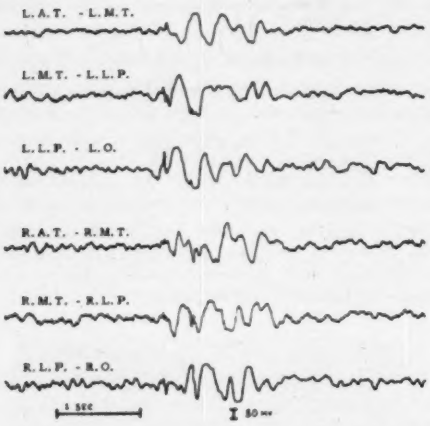

Fig. 4.—Electroencephalogram made on 5-year-old boy one year after recovery from moderately severe Rocky Mountain spotted fever which was treated with an antibiotic drug on fifth day of rash. No history of neurologic disturbance was obtained, and no neurologic abnormalities were observed in physical examination during acute illness or on reexamination. "Spikes" in center of tracing, which was made at onset of sleep, probably indicate brain damage in left hemisphere. Letters at left indicate leads.

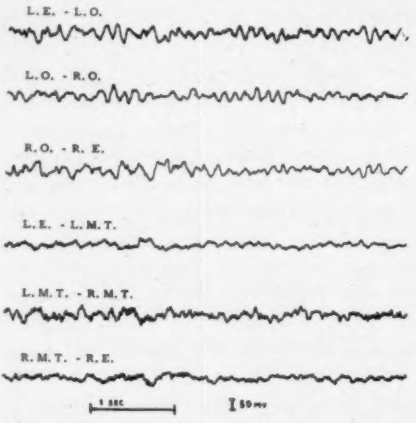

Fig. 5.—Electroencephalogram made on 9-year-old girl four years after recovery from severe rickettsial spotted fever, during which fever lasted more than 20 days. Clinical photographs made during the acute illness have previously been published.[3] During acute phase no neurologic abnormalities were detected, and spinal fluid was normal. At reexamination history of nervousness and emotional lability was obtained, but no abnormal neurologic signs were elicited. Tracing shows generalized six-per-second rhythm with focal fast activity in right hemisphere, suggesting widespread disturbance of cortical activity. Changes were considered borderline.

basic rhythm (one case). Only patients whose records showed these definite abnormalities were considered to have residual neurologic damage. In 12 additional patients the electroencephalograms showed borderline abnormalities which could have been within the limits of normal variability. These abnormalities consisted of slow or fast rhythm, minor irregularities of rhythm, or slight asymmetry. Thirteen records were clearly normal.

In summary, 21 of the 37 patients reexamined had neurologic abnormalities detected by history, physical examination, or electroencephalogram (Table).

Correlation of Electroencephalographic Findings with Other Features.—Clinical Severity of Illness: An attempt to correlate the electroencephalographic tracings with the clinical severity of the acute illness was inconclusive. Occasional patients whose disease was classified as mild or moderately severe showed abnormalities, and not all the patients with the severe disease had abnormalities on reexamination. Of the 15 patients who had neurologic abnormalities during the acute illness, 7 had clearly abnormal tracings on reexamination; for 4 patients the tracings were borderline and for 4 they were normal.

Residual Findings in Relation to Treatment

Treatment	No Residuals	Symptoms	Symptoms and Signs	Electroencephalographic Abnormalities: Alone	Symptoms	Signs	Symptoms and Signs	Total
Supportive	4	3	1	..	4	1	..	13
Serum and/or PABA	5	2	..	1	..	2	1	11
Antibiotic	7	3	..	2	..	1	..	13
Total	16	8	1	3	4	4	1	37

Duration of Fever: An analysis of the electroencephalographic tracings in relation to the duration of fever shows a high incidence of abnormal or borderline tracings in patients who had more than 10 days of fever after the appearance of rash. Of the four patients with fever for more than 20 days, three had abnormal electroencephalograms and one had a borderline tracing; one was mentally retarded, had positive neurologic signs, and suffered from convulsions.[5]

Treatment: When the electroencephalographic findings are correlated with the type of treatment, it is found that 5 of the 13 patients who were treated symptomatically with supportive measures alone had abnormal tracings; for 6 of these 13 patients, the tracings were borderline, and for only 4 were they normal (Table). The two patients who gave a history of convulsions developing after recovery had received only supportive therapy. Two additional patients who have been seen in our clinic because of convulsive disorders which followed Rocky Mountain spotted fever are not included in this series because they had been treated elsewhere during the acute illness; they, too, had received only supportive therapy. Eleven patients were treated with serum, paraaminobenzoic acid (PABA), or both. Four of these had abnormal electroencephalograms, three had borderline tracings, and four had normal records.

5. This is the patient referred to by Thomas and Berlin [4b]; when she was last seen six years after the acute illness, the residual findings were unimproved.

Of the 24 patients who received supportive therapy, serum, or paraaminobenzoic acid, 15 had residual neurologic abnormalities of some sort. It was our impression that these patients showed a greater tendency toward severe residual damage than did the patients who were given specific antibiotic therapy.

Of the 13 patients who were treated with aureomycin or chloramphenicol, 6 had some evidence of residual neurologic damage; 3 of these had only symptoms. For three the electroencephalogram was abnormal; four had borderine tracings, while six had normal records. Only one of the seven patients treated within the first three days of rash had an abnormal electroencephalogram on reexamination; two had borderline tracings, while tracings for four were clearly normal. Of the six patients treated between the fourth and the seventh day of rash, two had abnormal, two had borderline, and two had normal tracings.

The duration of fever was greatest in the group treated with supportive measures alone and shortest in the group given specific therapy. Consequently, it is difficult to distinguish between the direct effect of the antibiotic agent on the parasite and its indirect effect on the duration of fever in the prevention of these abnormalities.

Age of Patient: An analysis of the electroencephalographic findings in relation to the age of the patient shows that 19 patients were under 10 years of age at the time of follow-up; tracings were abnormal in 8 of these, borderline in 5, and normal in 6. In 13 patients between 11 and 20 years of age, two tracings were abnormal, five were borderline and six were normal. One patient between 21 and 30 and one between 41 and 50 had borderline tracings, and one between 31 and 40 had a normal tracing, while tracings for both patients over 50 were abnormal. The normal electroencephalogram varies with age, and this factor must be considered in interpreting tracings.[6]

COMMENT

Residual damage to the central nervous system is common following rickettsial spotted fever. It was possible to follow two-thirds of the patients who recovered from the acute illness during an eight-year period. Neurologic abnormalities have been detected by history, physical examination, or electroencephalogram in more than half of 37 patients reexamined at least one year after the completion of convalescence. It is unfortunate that data could not have been obtained for comparison before the onset of spotted fever in these patients, since it is possible that disturbances detected after recovery were present in some degree before the illness.

In only one patient was an electroencephalogram made during the acute phase of the illness. This was a 67-year-old woman who subsequently died of the disease in spite of antibiotic therapy. The electroencephalogram showed only borderline abnormalities (Fig. 6); at autopsy, the cerebral cortex grossly showed what were interpreted as petechial hemorrhages.[7] Microscopically, phagocytes containing blood pigment were found in the perivascular and subarachnoid spaces (Figs. 7 and 8). Microinfarcts and areas of gliosis with increased cellularity were also seen. Autopsies were performed on eight patients who died during the acute phase of the disease.

6. Gibbs, F. A., and Gibbs, E. L.: Electroencephalographic Changes with Age in Adolescent and Adult Control Subjects, Tr. Am. Neurol. A. **70**:154-157, 1944. Gibbs, F. A.: Cortical Frequency Spectra of Healthy Adults, J. Nerv. & Ment. Dis. **95**:417-426 (April) 1942.

7. Previously unpublished data on fatal cases observed in this clinic.

In six of these the brain was examined, and microscopic evidence of damage was found in all (Figs. 2, 3, 7, and 8).[7] It is apparent that lesions of the central nervous system occur in a high proportion of fatal cases.

The detailed data are difficult to interpret because of the great number of variables in this series. Rickettsial spotted fever varies widely in clinical severity. Differences in the virulence of strains recovered from various localities have been noted. Within any given area, the severity varies from year to year; this variation apparently is influenced by the weather, which affects the number of tick vectors rather than the virulence of the rickettsias. Variation within the same year is often noted, the severer attacks occurring early in the season. In any area the mortality rate is greatly increased in patients more than 40 years of age. It may be significant that abnormalities in the electroencephalogram were more frequent in the middle-aged and elderly patients in our series, but these abnormalities may have been the

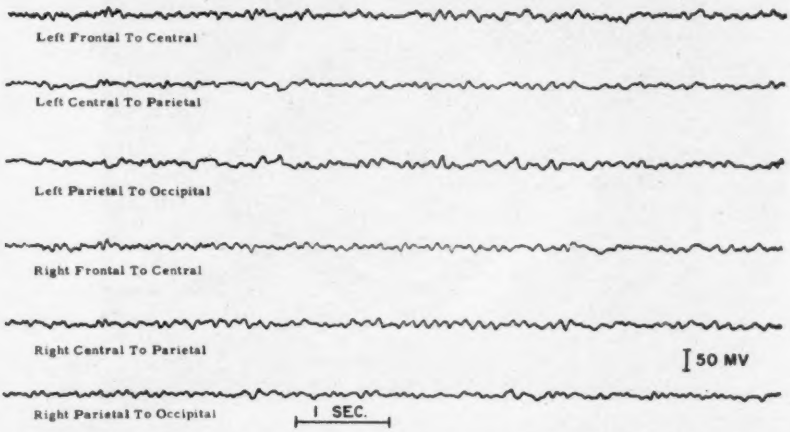

Fig. 6.—Electroencephalogram made on 67-year-old woman on sixth day of rash. Three days previously spinal fluid contained 7 red and 4 white blood cells per cubic millimeter; it contained 127 mg. of protein per 100 cc. and gave a 1 + Pandy reaction. Left pupil was dilated and did not react well to light. Neck was stiff, and Babinski sign on left was present. Oral antibiotic therapy was given, but patient died of peripheral circulatory collapse on 13th day of rash. Pathologic changes in brain are shown in Figures 7 and 8. Tracing shows generalized disturbance with unusual amount of six-per-second activity; it was interpreted as showing borderline abnormalities.

result of other febrile illnesses, of degenerative changes resulting from vascular disturbances, or of increasing age.

Factors Affecting the Development of Neurologic Sequelae.—Because of the small size of the groups involved (Table), it is also difficult to draw conclusions concerning the effect of various types of therapy in preventing neurologic sequelae. Three of these patients had been treated with sulfonamides before the correct diagnosis was made. These drugs are known to aggravate the clinical disease, but the number of patients treated with sulfonamides was too small to permit conclusions concerning their effect on the production of neurologic lesions.

Antirickettsial immune serum and paraaminobenzoic acid, though of some slight value as specific therapy if given before the third day of rash, are much less effective than antibiotics, and for the purposes of this discussion might be grouped with supportive therapy. It is our impression that patients treated with supportive measures alone are more likely to have residual neurologic lesions than are patients given adequate specific treatment with antibiotics. The use of aureomycin and chloramphenicol (all the patients in this series were treated before the introduction of terramycin) has greatly reduced the toxemia of the patients and the duration of the fever. In general, it would appear that the duration of fever, which serves as a measure of activity, is a more reliable prognostic index to the development of sequelae than is the clinical severity of the illness. The longer activity persists in the lesions, the more likely are granulomatous changes to occur (Fig. 3).

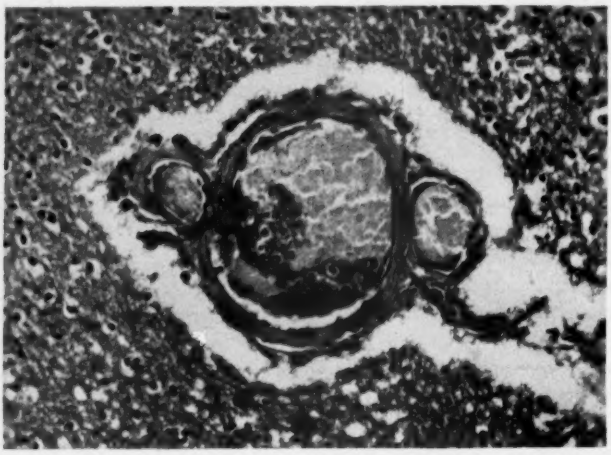

Fig. 7.—Photomicrograph of section of brain from 67-year-old woman whose electroencephalogram is reproduced in Figure 6. In dilated perivascular space phagocytes containing blood pigment can be seen. Neither large artery nor brain substance shows lesions of spotted fever.

Since the route of administration, dose, and duration of antibiotic therapy were variable, no detailed recommendations concerning the schedule of antibiotic therapy which will be most likely to prevent the development of sequelae can be made here. Antibiotics clear viable organisms from the circulation by the second day of treatment, and it is unlikely that many new lesions will develop after the rickettsias are eradicated from the blood.

The beneficial effect which the hormones corticotropin (ACTH) and cortisone are known to have in reducing disturbances resulting from arteriolar lesions in other diseases has recently been observed also in Rocky Mountain spotted fever.[8] A short

8. Arney, W. C.: Cortisone and Aureomycin in the Treatment of a Case of Rocky Mountain Spotted Fever, North Carolina M. J. **13**:20-22 (Jan.) 1952. Workman, J. B.; Hightower, J. A.; Borges, F.; Furman, E., and Parker, R. T.: Cortisone as an Adjunct to Chloramphenicol in the Treatment of Rocky Mountain Spotted Fever, read before the Southern Society for Clinical Research, Atlanta, January, 1952.

course of these hormones administered simultaneously with antibiotics has decreased the febrile period even more than antibiotic therapy alone. Whether such treatment will serve to reduce the extent or frequency of sequelae in the central nervous system is unknown.

It is difficult to determine how much residual neurologic damage may result from physiologic alterations which are not accompanied with anatomical changes. It has been our impression that abnormalities in function which result from alterations in the permeability of membranes are reversible with recovery. Certainly it is to be expected that any excessive hydration of nerve cells would have disappeared in the interval of more than a year which elapsed between the acute illness and the

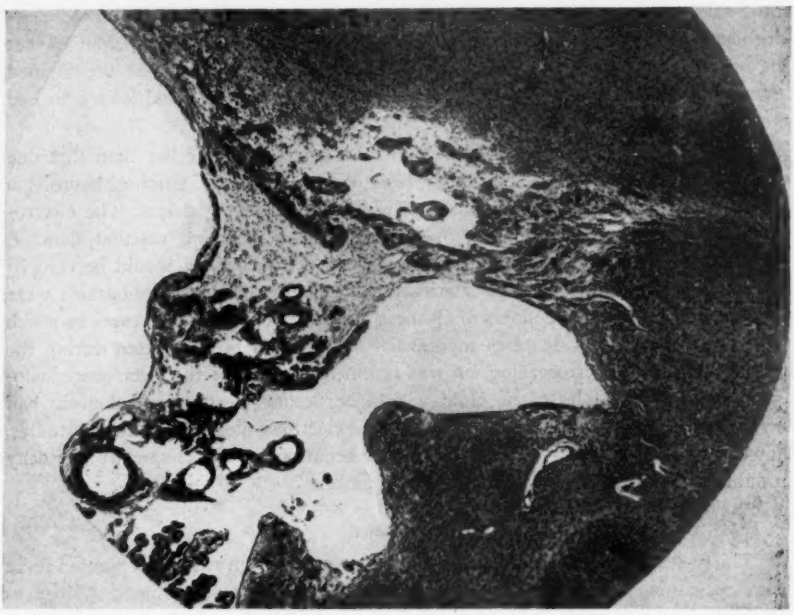

Fig. 8.—Photomicrograph of section of cortex from brain of patient illustrated in Figures 6 and 7. Pigment-containing phagocytes can be seen in meningeal spaces in center left portion of field. At upper portion of field nerve tissue is necrotic and is disappearing. At lower right cellularity of convolution is increased toward meningeal surface, as compared with deeper layers of brain at edge of section.

time of reexamination.[9] Careful supportive therapy, with close attention to protein and mineral metabolism, will reduce alterations in fluid distribution and may thus help to prevent possible damage resulting from excessive hydration of nerve cells.

Subsequent investigations may show that changes in the electroencephalogram such as we have observed to follow Rocky Mountain spotted fever (Figs. 4 and 5) sometimes follow other infectious diseases as well. The role of immune reactions

9. Harrell, G. T., Jr., and Aikawa, J. K.: Alterations in the Permeability of Membranes During Infections, J. A. M. A. **147**:232-238 (Sept. 15) 1951.

in altering the anatomical structure, as well as the permeability of blood vessels, is poorly understood. Many diseases, such as panarteritis and lupus erythematosus, in which antigen-antibody reactions are thought to play a major part are characterized by arteriolar lesions which in some instances are almost indistinguishable pathologically from those of rickettsial spotted fever.

At present, it is not known what effect fever alone, apart from the antigen-antibody reaction, would have on the electroencephalogram. Nine patients with fever, including three with rheumatic fever, two with lupus erythematosus, and one each with rheumatoid arthritis, a reaction to penicillin, serum sickness, and trichinosis, were studied with electroencephalograms during their illnesses.[10] Only the patient who had trichinosis (a disease which pathologically may be confused at times with panarteritis [11]) and clinical signs suggesting encephalitis showed electroencephalographic abnormalities, consisting of generalized irregular slow waves; the other patients all had normal tracings. These patients should be reexamined after an interval of years to be comparable with the group reported here who had recovered from spotted fever.

It is difficult to interpret the role which vascular damage other than that due to Rocky Mountain spotted fever might have played in the production of neurologic sequelae. The interpretation is particularly difficult in older patients. The electrocardiogram may serve as another objective means of detecting vascular damage, though its interpretation, like that of the electroencephalogram, would be complicated by the factors of age and arteriosclerotic changes. Electrocardiograms were not made during the acute illness of all these patients; however, in all cases in which electrocardiographic evidence of myocardial damage had been detected during the febrile phase, the electrocardiogram was repeated at the time the electroencephalogram was made. At least one child with a borderline electroencephalogram had definite evidence of myocardial damage in the electrocardiogram. Autopsy studies, however, have shown that myocardial damage occurs much less frequently in Rocky Mountain spotted fever than does cerebral damage.

SUMMARY

Thirty-seven patients of all ages who had recovered from rickettsial spotted fever were reexamined one to eight years after the acute phase of the illness. Evidences of residual neurologic damage were sought in the history, physical examination, and electroencephalogram.

At the time of reexamination 21 of the 37 patients had some type of neurologic sequela. Fourteen patients gave a history of symptoms related to the central nervous system; 6 had neurologic signs, and 12 had clearly abnormal electroencephalograms. Twelve additional patients had borderline electroencephalographic abnormalities. The electroencephalogram was abnormal in a high proportion of patients who had had fever for more than 10 days.

10. Unpublished preliminary data collected in this clinic. Additional data on patients with infections and with fever from metabolic causes in which antigen-antibody reactions would not occur are desirable.

11. Reimann, H. A.; Price, A. H., and Herbut, P. A.: Trichinosis and Periarteritis Nodosa: Differential Diagnosis; Possible Relationship, J. A. M. A. **122**:274-279 (May 29) 1943.

Of 15 patients who had been observed to have abnormal neurologic signs during the acute illness, 3 had residual signs, and 2 were having convulsions. Seven of these 15 patients had abnormal electroencephalograms; 4 had borderline tracings, and 4 showed no electroencephalographic changes.

Of 24 patients who received supportive therapy alone (including those treated with antiserum or paraaminobenzoic acid), 14 had neurologic sequelae of some sort; these sequelae were severer than those seen in patients who received antibiotic therapy. Of the 13 patients treated with antibiotics, 6 had evidences of residual neurologic damage. Only one of seven patients receiving antibiotics within the first three days of rash had an abnormal electroencephalogram.

Eight patients who died during the acute illness were examined at necropsy. Microscopic evidence of damage was found in all six patients in whom the brain was examined. In these patients microinfarcts or granulomas were found adjacent to cerebral blood vessels.

CONCLUSIONS

Damage to the central nervous system is common in spotted fever. In some patients the acute changes are reversible with convalescence; in others residual damage persists longer than a year and may be assumed to be permanent.

HISTOPLASMOSIS

Three Fatal Cases with Disseminated Sarcoid-like Lesions

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THE THREE fatal cases of histoplasmosis with disseminated sarcoid-like lesions which are reported in this paper serve as evidence for the view that subacute or chronic histoplasmosis may, in some instances, exhibit certain clinical and pathologic features often regarded as diagnostic of sarcoidosis.

In recent years it has become clear that the clinical manifestations evoked by Histoplasma capsulatum are as protean as those of tuberculosis. Histoplasmosis may be localized or generalized; often it is localized for months or years, eventually becoming generalized. It may be acute, subacute, recurrent, or chronic, and it may be severe, mild, or completely asymptomatic.[1] Almost any organ or tissue may be involved, but lesions are most frequently seen in the lungs, lymph nodes, spleen, liver, intestine, adrenal glands, bone marrow, skin, and naso-oral cavity.

The lesions of histoplasmosis are almost as varied as the clinical picture. They show every gradation of granulomatous reaction from pure macrophagic response to fibrotic, hyaline, or calcific repair. Tuberculoid granulomas, with or without necrosis, may occur, as well as nonspecific diffuse inflammation. In fatal cases these lesions may be found in many different combinations. Frequently there is necrosis in one or two organs only, with noncaseous "tubercles" in the lymph nodes, spleen, lungs, liver, and other organs. Recently, in a review of 20 cases of histoplasmosis, disseminated tuberculoid lesions were noted in 6, and in none of these was there reason for believing that concomitant infection with the tubercle bacillus or any other organism was present. It should be emphasized that fungi are rarely identified in these noncaseous, focal lesions.

Although the resemblance of the tuberculoid lesions of histoplasmosis to sarcoid was suggested by Henderson, Pinkerton, and Moore [2] in 1940, their possible con-

From the Armed Forces Institute of Pathology and the St. Louis University School of Medicine.

1. (*a*) Reimann, H. A.: Histoplasmosis, in Musser, J. H., and Wohl, M. G.: Internal Medicine, Its Theory and Practice, Ed. 5, Philadelphia, Lea & Febiger, 1951, p. 388. Furcolow, M. L.: (*b*) Tuberculin Negative, Histoplasmin Positive, Disseminated Pulmonary Calcification, Postgrad. Med. **8**:15-20, 1950; (*c*) Further Observations on Histoplasmosis: Mycology and Bacteriology, Pub. Health Rep. **65**:965-994, 1950.

2. Henderson, R. G.; Pinkerton, H., and Moore, L. T.: Histoplasma Capsulatum as a Cause of Chronic Ulcerative Enteritis, J. A. M. A. **118**:885-880, 1942.

fusion with sarcoidosis was first emphasized by Reimann and Price [3] in 1949. The case reported by these authors was that of a 28-year-old Negro woman, who seven years before death showed the characteristic signs and symptoms of sarcoidosis: papular and nodular cutaneous lesions, thoracic pain, chills, sweating, leucopenia, and, by roentgenogram, nodular densities in both lung fields. Four years before death, biopsy of a cutaneous lesion showed granulation tissue and epithelioid tubercles without caseation "suggestive of sarcoidosis." The pulmonary lesions multiplied; the spleen became palpable, and the characteristic roentgenologic evidence of sarcoidosis became apparent in the phalanges. After H. capsulatum was identified on biopsy sections from an ulcer in the larynx, study of material from the cutaneous lesion obtained four years previously also revealed H. capsulatum. At autopsy, granulomatous lesions were present in the lungs, liver, lymph nodes, and adrenal glands. Fungi were identified only in the adrenal glands. In a second publication,[4] these authors made brief mention of a similar case submitted by Israel and DeLamater, in which sarcoidosis and histoplasmosis appeared to have been associated.

The three fatal cases of histoplasmosis with sarcoid-like lesions to be reported here were encountered in a review of material in the files of the Armed Forces Institute of Pathology. Originally the fungi had been overlooked in Cases 1 and 3, diagnosed and reported [5] as instances of disseminated sarcoidosis. The present study suggests that they may be examples of uncomplicated histoplasmosis. In Case 2 the correct diagnosis of histoplasmosis was made at autopsy, although four months prior to death the patient was thought to have sarcoidosis because of the prominence of epithelioid tubercles in an excised axillary lymph node.

REPORT OF CASES

CASE 1 (AFIP Accession 119987).—*History.*—The patient was a 30-year-old white man who had served in the Armed Forces for three years before becoming ill. Eight months before death, while he was stationed at an Army camp in Indiana, his appetite gradually decreased and not long afterward he noticed afternoon fatigue, perspiration, and fever. Two months later he entered the hospital; enlarged superficial lymph nodes were palpable, and calcified abdominal nodes were demonstrated by roentgenologic studies. However, no foci of infection could be found in any area of the body convenient to examination, and results of specific tests, including blood cultures and agglutinations for paratyphoid and Brucella organisms, remained negative.

Four months after the onset of his illness, biopsy of a right inguinal node showed discrete hard tubercles "compatible with low-grade miliary tuberculosis or sarcoidosis" (Fig. 1). Guinea-pig inoculations and cultures of the lymph nodes yielded no organisms. On roentgen examination no pulmonary changes were demonstrable, and the only bony changes were in the metacarpals, where slight demineralization was evident at the level of the epiphyses, and in the carpals, which also contained areas of decreased density. During hospitalization the patient's afternoon temperature elevation to 100 to 103 F. remained unchecked by penicillin therapy. Because of hypotension and fatigue, adrenal cortex extract therapy was instituted but occasioned no improvement. During the last three months of life, the patient became increasingly anemic and the spleen was palpable and tender. He continued to lose weight steadily and died eight months after the onset of illness.

3. Reimann, H. A., and Price, A. H.: Histoplasmosis in Pennsylvania: Confusion with Sarcoidosis and Experimental Therapy with Bacillomycin, Pennsylvania M. J. **52**:367-371, 1949.

4. Reimann, H. A., and Price, A. H.: Histoplasmosis Resembling Sarcoidosis, Tr. A. Am. Physicians **62**:112-115, 1949.

5. Ricker, W., and Clark, M.: Sarcoidosis: A Clinicopathologic Survey of 300 Cases, Including 22 Autopsies, Am. J. Clin. Path. **19**:725-749, 1949.

Postmortem Examination.—Gross Observations: The lungs were soft and crepitant except for the firm, grayish-white dependent portions. Beneath the visceral pleura of the right lower lobe was a partly calcified nodule, 6 mm. in diameter, with a soft caseous center. The regional nodes contained similar calcified foci, 4 to 8 mm. in diameter. The spleen was enlarged to 575 gm. The abdominal nodes, also enlarged, were marked by occasional grayish-white foci resembling tubercles. Of the remaining viscera, gross abnormalities were noted only in the adrenal glands. The combined weight of these organs was 95 gm., and both were transformed into swollen, nodular, caseous masses. The brain was not examined.

Microscopic Observations: Microscopic examination revealed granulomatous inflammation in the lymph nodes, lungs, liver, spleen, kidneys, and adrenal glands. Sections of the intestine were not included with the material.

Lymph nodes: The nodular clumps of solidly packed epithelioid tubercles seen in the node removed surgically were not completely duplicated. Some of the enlarged nodes showed merely lymphoid hyperplasia and dilated sinusoids loosely filled with histiocytes.

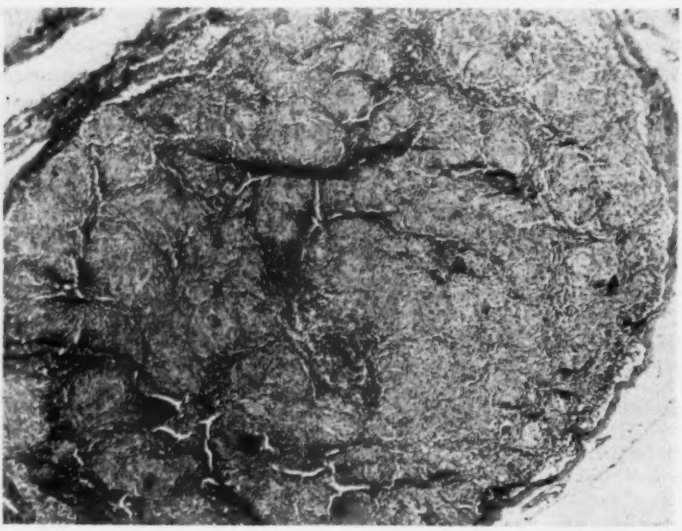

Fig. 1 (Case 1 [AFIP Accession 119987]).—Masses of epithelioid tubercles in an inguinal lymph node removed four months before death; × 75.

In other nodes the cortex contained isolated giant cells, occasionally accompanied with histiocytic satellites, at times attaining tuberculoid definition. In such nodes small accumulations of homogeneous eosinophilic material were noted. Occasional nodes were almost completely replaced by this substance, which did not react like amyloid to Congo red, and in these nodes the epithelioid tubercles tended to be outlined by bands of the homogeneous material. Schaumann bodies were not uncommon and were characterized by concentric mineral deposits about doubly refractile crystals (Fig. 2). In a lymph node removed from the tracheobronchial region, the normal components had been replaced by hyaline fibrous tissue in which were scattered precipitates of calcium. The periphery of this node retained a thin rim of viable mononuclear cells occasionally clustered around giant cells in quasitubercle formation. No organisms and no caseous necrosis were seen in the lymph nodes.

Lungs: Diffuse edema, congestion, and interstitial infiltration of the mononuclear type were the changes seen uniformly in the lungs. Small disseminated tubercles were present, resulting in nodular swellings of the alveolar, bronchiolar, and vessel walls. A lymphocytic reaction accom-

panied the tuberculoid lesions, which, when healed, were visible either as tiny, oval whorls of fibrous tissue, sometimes thickening the vascular wall, or as tiny constricting scars in the terminal segment. There was no tendency to confluence or caseation of the tubercles, and no organisms were noted.

Liver: Single tubercles similar to those seen in the lung were scattered throughout the liver. These tended to localize in the portal areas, where they were accompanied with a lymphocytic reaction. Healing of these areas resulted in an increase of portal fibrous tissue and accentuation of the hepatic lobular architecture. No caseation or necrosis of the tubercles was noted; no organisms were seen.

Spleen: The splenic pulp was congested and diffusely altered by isolated and confluent epithelioid tubercles (Fig. 3*A*). Schaumann and asteroid bodies were frequent. No organisms were seen.

Adrenal glands: Both glands were entirely necrotic except for a few recognizable cortical cells with areas of diffuse necrotizing granulomatous inflammation interspersed at the periphery.

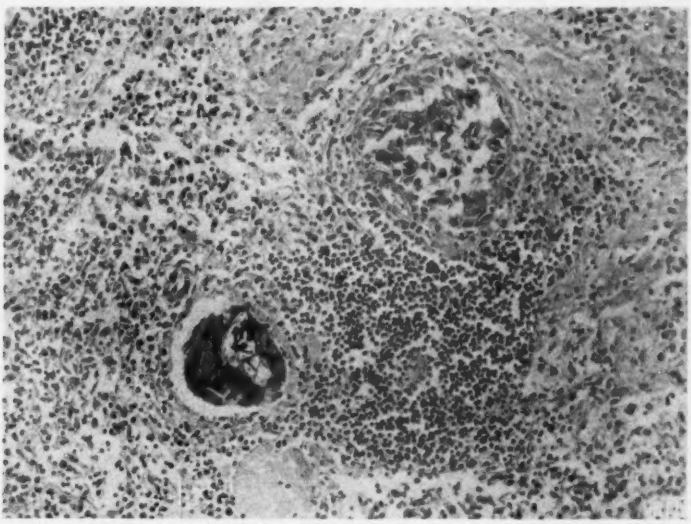

Fig. 2 (Case 1).—Sarcoid-like lesions in an abdominal lymph node studied post mortem. Note the Schaumann crystals and the deposits of protein material outlining follicles; × 125.

Viable mononuclear cells and lymphocytes remained in the central, more vascular portions of the gland. Here, hematoxylin and eosin stains showed a few macrophages swollen by tiny globular bodies 2 to 4 μ in diameter, resembling H. capsulatum (Fig. 3*B*). In sections stained with periodic acid, the central caseous mass was seen to be packed with large aggregations of Histoplasma organisms, overlooked in the standard preparations.

Case 2 (AFIP Accession 121022).—*History.*—The patient was a 26-year-old white Tennessee farmer who had served in the Army for eight months before he was admitted to the hospital. His illness had begun three years earlier with bouts of chills and fever. The attacks lasted three or four days and recurred at intervals of five to six weeks. Two weeks before admission the persistent temperature elevation was accompanied with anorexia and vomiting, weakness, and weight loss.

Examination revealed no definite signs other than slight elevation of temperature and mild degree of dehydration. However, 12 hours after admission the patient's temperature rose suddenly to 103.4 F. and continued to fluctuate irregularly until death. Within the first week the

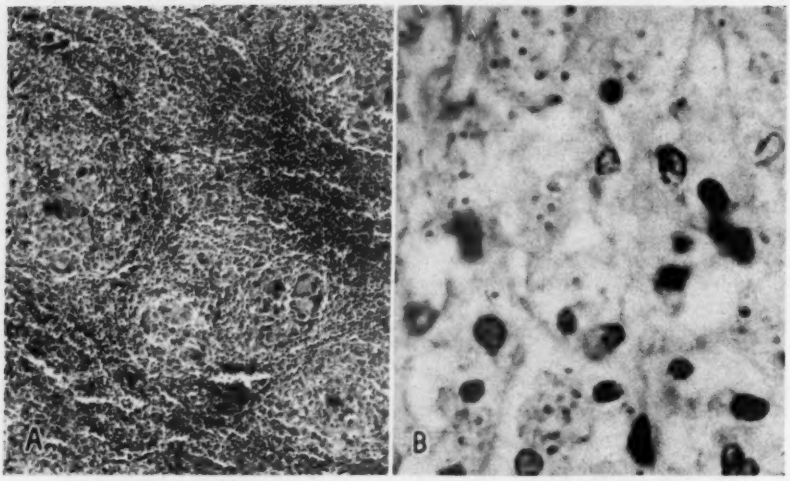

Fig. 3 (Case 1).—*A*, epithelioid tubercles diffusely scattered throughout the enlarged spleen; × 125. *B*, viable Histoplasma spores in macrophages surrounding the caseous area in the adrenal gland; × 825.

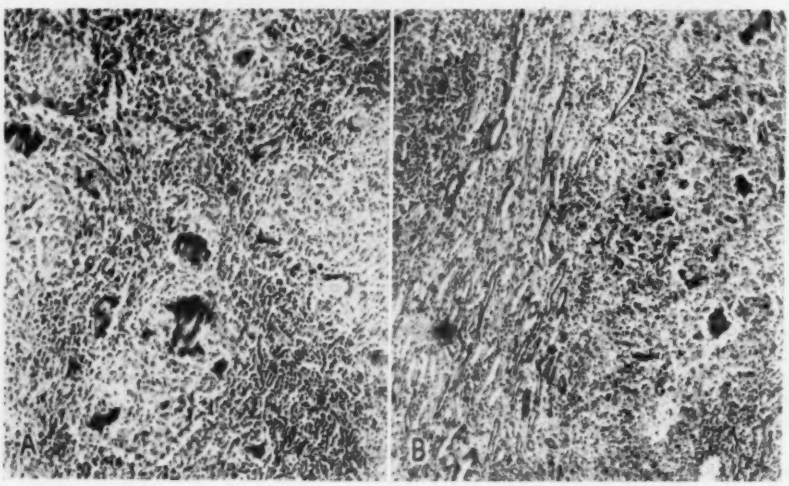

Fig. 4 (Case 2 [AFIP Accession 121022]).—*A*, epithelioid tubercles in an axillary lymph node removed four months before death; × 275. *B*, kidney: diffuse histoplasmic nephritis, with caseous and noncaseous focal lesions; × 205.

spleen and cervicoaxillary lymph nodes became palpable. Laboratory examination, including blood cultures, failed to demonstrate evidence of specific parasitic, mycotic, or bacterial infection. Biopsy of an axillary node was performed, and the diagnosis of "sarcoid" was established on the basis of the histologic examination (Fig. 4*A*). Extensive roentgenologic studies of the skeletal system showed no abnormalities. Intradermal testing with first- and second-strength purified protein derivative produced no reaction. Elevated eosinophile counts and sedimentation rates were the only positive accessory findings.

During the patient's stay in the hospital, the spleen continued to enlarge, ultimately reaching the midline. The patient became extremely weak, lethargic, and emaciated. He died four months after admission.

Postmortem Examination.—Gross Observations: Ovoid scars, as much as 6 mm. in length, extended superficially beneath the pleura of both pulmonary apices. Several small calcified subpleural nodules, 2 or 3 mm. in diameter, were noted in both the right and the left lungs. An enlarged tracheobronchial node was apparently replaced by fibrous tissue. The greatly enlarged spleen (1,204 gm.) was firm and reddish-purple, and its tense surface was interrupted by eight small, wedge-shaped, white necrotic areas. The mesenteric nodes were uniformly enlarged, and the cut surfaces of some revealed tiny foci of caseous necrosis. The mesenteric lymph node enlargement corresponded to the distribution of pinpoint ulcers throughout the entire length of the small intestine. The medulla of each kidney was focally altered by scattered soft white areas of inflammation. The enlarged right and left adrenal glands weighed 35 and 45 gm., respectively. The capsules were thickened and separated from the irregularly necrotic central portions by a thin golden rim of grossly intact cortex.

Microscopic Observations: The most prominent changes were the tuberculoid lesions in the lymph nodes, lungs, gastrointestinal tract, liver, spleen, and adrenal glands. Additional focal granulomas were noted in the thymus, heart, pancreas, kidneys (Fig. 4*B*), and pons. Organisms resembling H. capsulatum were identified only in the organs where caseation and necrosis characterized the reactions.

Lymph nodes: The axillary node removed surgically showed discrete epithelioid tubercles, sometimes formed around giant cells. Although no Schaumann bodies were noted, asteroid bodies were frequent, and large clefts in the cytoplasm suggested the presence of fat. Perifollicular deposits of homogeneous protein material (so-called paramyloid) enhanced the resemblance to the sarcoid reaction. In one portion of the node the tubercles lost their distinctness as they became matted together by interlacing strands of collagen. These changes were not duplicated in the enlarged nodes removed at necropsy; most of them were merely hyperplastic or contained only occasional giant cells and reticuloendothelial cells. In the abdominal nodes, areas of caseous necrosis replaced portions of the node. Calcium deposits and necrotic debris gave a faint blue cast to the periphery of such areas when periodic acid stains were required to demonstrate the organisms (Fig. 5). A few macrophages in the viable portions contained organisms which were clearly outlined in the hematoxylin and eosin preparations.

Lungs: At the pulmonary border of the pleural scars were noncaseating satellite tubercles, and similar lesions were scattered throughout the lung parenchyma. Organisms were distinguished with difficulty in rare macrophages. The focal areas of atelectasis and emphysema with moderate cystic change could be correlated with the presence of fibrosis from healing of the small tubercles.

Liver: This organ was greatly altered by a diffuse and focal granulomatous process involving the portal areas as well as the parenchyma. Almost all the tubercles were of a similar age and consisted of giant cells surrounded by a zone of reticuloendothelial cells, lymphocytes, and plasma cells. Some tubercles had reached the epithelioid stage, and others had progressed to healing. Where this occurred in the portal areas, the increased fibrosis led to accentuation of the lobular architecture and the appearance of early cirrhosis. No organisms were seen in the liver.

Spleen: The spleen was congested and almost entirely replaced by the reacting inflammatory cells which were grouped in poorly formed tubercles composed of giant cells, epithelioid cells, lymphocytes, eosinophiles, and plasma cells. At the centers of some tubercles were tiny areas of true suppuration, and at the periphery of others were deposits of amorphous protein material. Organisms resembling H. capsulatum were massed at the periphery of the large necrotic areas noted grossly.

Adrenal glands: These organs were similar in appearance to those described in Case 1. It is noteworthy that the Bauer and periodic acid-Schiff preparations revealed enormous numbers of organisms which were otherwise visible only as faint bubbles in the caseous portions of the gland; with hematoxylin and eosin stains intracytoplasmic fungi were seen in cortical cells at the periphery of each gland.

Case 3 (AFIP Accession 121747).—*History.*—In May, 1943, this 54-year-old white man was examined for retirement and was returned to duty with a diagnosis of coronary artery disease. His past history included a tour of duty in Panama, 1918-1919, and two previous hospital admissions for arthritis of the right wrist, when hepatomegaly was noted on the patient's record. In March, 1944, the patient became progressively weak, lost weight, suffered from vague gastrointestinal symptoms, and had two hemorrhages of undetermined source. When he was admitted to the hospital in August, 1944, physical examination revealed questionable pigmentation of the oral mucous membrane. Although the patient had previously had mild hypertension, blood-

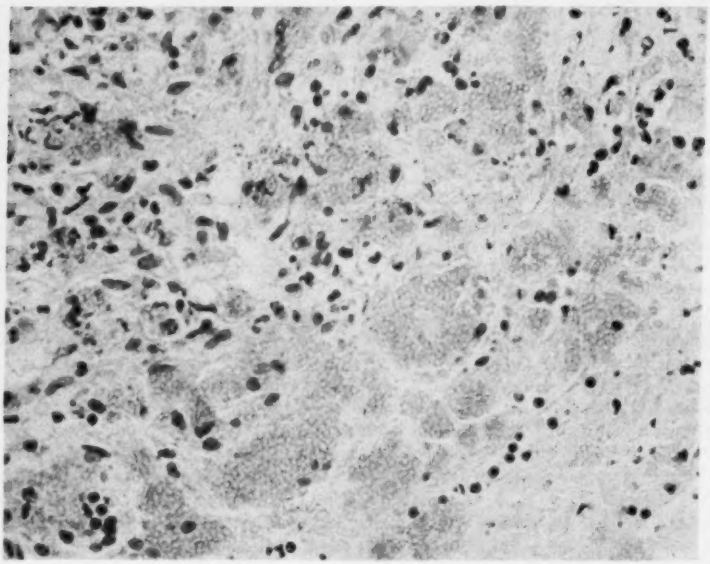

Fig. 5 (Case 2).—Clumps of organisms, H. capsulatum, are outlined by periodic acid-Schiff stains in the necrotic portions of an abdominal lymph node; × 525.

pressure readings were now reported at 92/50 and 105/70. The tentative diagnosis of Addison's disease was later supported by demonstration of hypoglycemia, dehydration, and elevated blood nonprotein nitrogen. The serum proteins, which initially reached 9 gm. per 100 cc., were later recorded at 5.6 gm., with an albumin-globulin ratio of 1.65. Blood chloride and sodium determinations were not done. Blood-pressure readings on several occasions during the subsequent course of the illness were 60/40, 70/40, and 40/20. Accessory clinical examinations also demonstrated urine of low specific gravity (1.010 to 1.012) and abnormal sulfobromophthalein (bromsulphalein®) sodium liver tests. The demonstration of gallstones and the hepatosplenomegaly resulted in the diagnosis of biliary cirrhosis.

A small circumscribed ulcer on the right lateral portion of the patient's tongue was thought to be carcinoma, and he was transferred in September, 1944, to another hospital for radiation therapy. He continued to lose weight and to suffer from epigastric pain, indigestion, and nausea. Dehydration persisted even though fluids were constantly administered intravenously. Ten days after transfer, after an episode of vomiting of coffee-ground material, the patient expelled

copious black liquid stools, became acidotic, began to breathe irregularly, and lapsed into coma. He died approximately seven months after the onset of illness.

Postmortem Examination.—Gross Observations: In addition to the ulcerated lesion on the tongue and the small gallstone suspected clinically, a duodenal ulcer was found to have eroded into the pancreaticoduodenal artery, thus causing the hemorrhage. The remaining viscera showed signs of a generalized infection. The right and left lungs, weighing 1,260 and 940 gm., respectively, were marked by bilateral apical, subpleural, and pleural fibrosis. Beneath the pleura of the right lower lobe was a small, firm white nodule, which together with the scarred hilar node suggested a primary tuberculous complex. The impression of tuberculosis was strengthened by the multiple, small, thick-walled cavities honeycombing the right apex. Grayish-white miliary lesions were scattered throughout the liver, spleen, kidneys, and the enlarged abdominal lymph nodes. In addition, the enlarged liver (3,100 gm.) showed fine perilobular fibrosis in the pattern characteristic of biliary cirrhosis. The spleen weighed 1,300 gm., was firm, and contained small tubercles with some confluence but no necrosis. The right and left adrenal glands, weighing 192 and 110 gm., respectively, were soft, necrotic, and caseous (Fig. 6*A*). Little identifiable cortex remained.

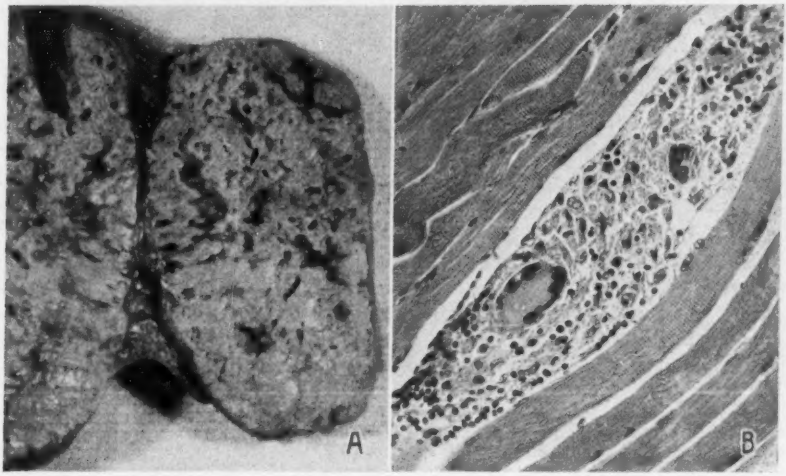

Fig. 6 (Case 3 [AFIP Accession 121747]).—*A*, an adrenal gland has been replaced by a large mass of caseous tissue. *B*, granulomatous lesions were present in striated muscle. Similar changes were seen in the heart; × 275.

Smears and cultures for fungi and tubercle bacilli were negative. Extensive cultures of other lesions failed to show bacteria or fungi, and injection of material from the lung into guinea pigs produced no disease.

Microscopic Examination: In addition to the sites noted in Cases 1 and 2, microscopic examination revealed granulomatous inflammation in the tongue, pectoral muscle (Fig. 6*B*), bone marrow, and gastric mucosa. The central nervous system showed no anatomical alterations.

Lungs: Diffuse chronic inflammation and fibrosis of the lungs were of nonspecific type. Organisms were not clearly defined in the focal areas of caseation with central cavity formation and peripheral fibrosis. Scattered granulomatous lesions were present (Fig. 7*A*).

Heart: The predilection of the organisms for localization around vessels was apparent. In the heart the granulomas tended to occur in the form of spindle-shaped accumulations of large mononuclear cells replacing muscle fibers. When located perivascularly, healed lesions of this description remained as elongated collars of fibrous tissue. There was occasional epithelioid and tuberculoid differentiation of mononuclear cells. No organisms were identified.

Liver: Severe chronic granulomatous inflammation in the portal areas was sometimes typified by giant cells and definite tubercle formation. Associated features were congestion, fatty alteration of parenchymal cells, and lobular regeneration.

Adrenal glands: These organs had undergone the same changes as described in Cases 1 and 2. The cortical tissue was entirely necrotic. At the margins of the caseous portions was a layer of acute inflammation and edema merging with a zone of granulomatous inflammation where numerous organisms were seen within cells of the adrenal cortex and macrophages. Periodic acid stains revealed clumps of apparently dead fungi within the caseous material (Fig. 7*B*). As in the other cases, the bulky caseous material representing the adrenal glands was composed in many areas almost entirely of empty capsules of H. capsulatum. Only peripherally, within well-preserved cells, did the fungi show normal internal structures.

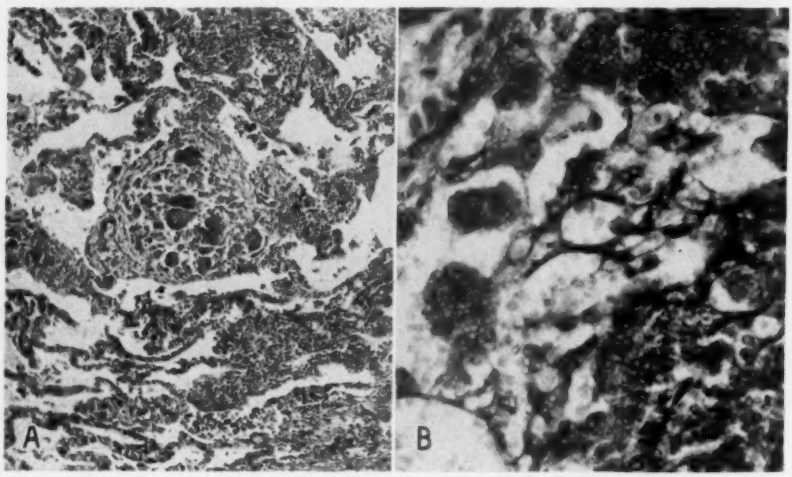

Fig. 7 (Case 3).—*A*, focal tubercles are scattered in the lung, which also shows diffuse mononuclear interstitial reaction; × 75. *B*, Histoplasma organisms are massed in caseous portions of the adrenal gland. Periodic acid-Schiff stain; × 525.

COMMENT

Longcope [6] described sarcoidosis as

> . . . a chronic, relapsing infection of the reticulo-endothelial system, usually benign but widely distributed throughout the body, affecting particularly the lymph node, lung, spleen, skin, bone marrow, and liver and characterized by solitary and conglomerate collections of epithelioid cells arranged in tubercle-like formations.

All the cases described in this report were, until the time of their reevaluation, thought to be examples of sarcoidosis. Presumably this was because of the following findings: numerous discrete epithelioid tubercles in excised lymph nodes or in the viscera removed at autopsy; the presence of Schaumann bodies and asteroid bodies, common in sarcoidosis but not diagnostic of that entity; negative tuberculin skin tests in the presence of a histologic picture resembling sarcoid; splenomegaly; bone

6. Longcope, W. T.: Sarcoidosis (Besnier-Boeck-Schaumann Disease), in Christian, H. A.: Oxford Medicine, New York, Oxford University Press, 1949, Vol. 4, Pt. 1, p. 44.

changes (Case 1); reversed albumin-globulin ratio (Case 3). The identification of organisms in Case 2 finally led to the correct diagnosis by autopsy, but Cases 1 and 3 were coded as examples of sarcoidosis complicated by tuberculosis.

These data may be interpreted in several ways: 1. The association of histoplasmosis and sarcoid-like lesions may be regarded as purely fortuitous. 2. If the previous diagnosis of sarcoidosis is correct, it may be supposed that the sarcoidosis lowered resistance to the fungous infection. 3. The lesions of histoplasmosis in tissue with effective immunity may simulate or even duplicate those of sarcoidosis.

While neither the first nor the second possibility can be arbitrarily excluded,[7] we regard the third explanation as most strongly supported by the evidence, especially since previously reported descriptions of histoplasmosis have embraced all the changes noted in these cases.[8]

For these reasons, it is worth while to emphasize the distinction between the disease sarcoidosis and the histologic change often called "sarcoid lesions."[9] It is from this capacity of H. capsulatum to stimulate the formation of epithelioid tubercles that most of the confusion stems to result in the misdiagnosis of sarcoid. Since this ability is shared by many other granulomatous excitants, Mycobacterium tuberculosis, Mycobacterium leprae, Blastomyces dermatitidis, and Brucella, these cases suggest that even extensive replacement of lymphoid tissue by epithelioid tubercle is not an adequate basis for the diagnosis of sarcoidosis unless the accompanying clinical picture is entirely characteristic and subsequent histologic studies show little variation from the usual morphologic pattern. It should be stressed that when the initiating organism is small or lacks a readily stained capsule, as in tuberculosis or histoplasmosis, it cannot be easily demonstrated in non-necrotizing epithelioid lesions. Consequently extensive search of areas of epithelioid reaction naturally fails to reveal organisms and failure to demonstrate organisms does not indicate "sarcoidosis."

Even in cases in which the clinicopathologic features are more in keeping with the disease sarcoidosis (Cases 1 and 3) and in previously reported cases, it is not unlikely that the histologic lesions thought to be characteristic may represent only a particular pattern of immunologic response to which Teilum[10] has given the name "allergic hyperglobulinosis." If this is so, a relation between sarcoidosis and histoplasmosis may exist in the manner suggested by some workers as probable in certain cases of tuberculosis.[6] There are insufficient laboratory data to justify such a theory, but it is not inconceivable that fungi entering the blood stream from active focal lesions in the lungs, adrenal glands, or elsewhere may be carried to lymph nodes, skin, or other organs. There, because of tissue immunity, they may be rapidly killed, setting free antigen which results in the production of noncaseating lesions of the sarcoid type. If the active focal lesions eventually heal or become quiescent, a prolonged and relatively benign clinical course with periodic exacer-

7. Murrau, J. F., and Brandt, F. A.: Histoplasmosis and Malignant Lymphoma, Am. J. Path. **27**:783-799, 1951.

8. Reimann.[1a] Henderson and others.[2] Reimann and Price.[8]

9. Freimann, D. G.: Medical Progress: Sarcoidosis, New England J. Med. **239**:664-671; 743-749, 1948.

10. Teilum, G.: Allergic Hyperglobulinosis and Hyalinosis (Paramyloidosis) in the Reticulo-Endothelial System in Boeck's Sarcoid and Other Conditions: Morphologic Immunity Reaction, Am. J. Path. **24**:389-407, 1948.

bations may ensue. The histochemical studies described showed that dead fungi without internal structure can be identified only by special stains which color their capsules. These studies would indicate that some of those cases with the diagnosis of sarcoidosis may be due to the organism H. capsulatum.

The splenomegaly in our three patients is of particular interest. In this connection Kay [11] has reported four cases of presumable splenic sarcoidosis in which surgical removal of the huge spleens has been followed by relatively good health for many years. He suggested that the lesion in his cases may be identical with the so-called Stengel-Wolbach sclerosis. The spleens in our cases greatly resembled those in the cases reported by Kay, and it is conceivable that his differ from ours only in that the foci from which dissemination of fungi occurred became quiescent or healed. Spleens so greatly enlarged by mycotic granulomatous processes would not be expected to return to anything like normal size, even if all fungi in the body were killed and completely resorbed.

Only after more cases are added to those in the literature and more extensive laboratory data are available will this problem be resolved. It seems possible that the use of periodic acid stains in biopsy material from a large series of cases of "sarcoidosis" might reveal the presence of fungous capsules in an occasional case. These fungi, which are no longer viable, would, of course, not cause infection in animals. Skin tests with fungous antigens may also furnish evidence of value. The antigen present in lymph nodes which is responsible for the Kveim test might be tested in guinea pigs with histoplasmosis and other fungous infections. Until such studies have been carried out, it would be useless to hazard a guess concerning the frequency with which H. capsulatum may cause the lesions that lead to a diagnosis of sarcoidosis. We agree with those who hold that neither the stellate inclusions nor the Schaumann bodies within giant cells are specific for sarcoidosis or for any other single disease.

In geographic distribution, sarcoidosis appears to differ from histoplasmosis, the former being commonest in the southeastern United States and the latter in the midwestern states. This suggests that testing with blastomycin, histoplasmin, and coccidioidin of patients suspected of having sarcoidosis might furnish evidence concerning the role that other fungi may play in diseases which clinically and pathologically simulate sarcoidosis.

These three cases present a second diagnostic problem which results from the histologic resemblance of certain lesions to tuberculosis. In two of these cases, consulting pathologists interpreted the caseous adrenal glands as indication that patients with sarcoidosis had died of tuberculosis. In one case the caseous cavities in the lung were also interpreted as tuberculosis, although no organisms were subsequently demonstrated. Other similarities to tuberculosis reported in the literature have included pulmonary calcification,[1b] the primary complex or Ghon lesion,[12] and pulmonary cavitation.[13] Consequently, these observations indicate that

11. Kay, S.: Sarcoidosis of the Spleen: Report of 4 Cases with a 23 Year Follow-Up in One Case, Am. J. Path. **26**:427-443, 1950.

12. Schulz, D. M.: A Partially Healed Lesion in a Case of Generalized Histoplasmosis, Arch. Path. **50**:457-463, 1950.

13. Hodgson, C. H.; Weed, L. A., and Clagett, O. T.: Pulmonary Histoplasmosis: Summary of Data on Reported Cases and a Report on 2 Patients Treated by Lobectomy, J. A. M. A. **145**:807-813, 1951.

caseous adrenal necrosis should not be assumed to be of tuberculous origin unless sections of the involved tissue are studied with special stains capable of demonstrating mycotic as well as acid-fast organisms.

SUMMARY

Sarcoid-like lesions were present in lymph nodes, lung, spleen, liver, and other organs in three cases of fatal histoplasmosis. The cause of death in every case was bilateral mycotic caseation of the adrenal glands. In the caseous material periodic acid stains demonstrated huge numbers of Histoplasma organisms which could not be identified in sections stained in the routine way.

Although the possibility cannot be entirely excluded that these cases may represent the concurrence of two diseases, sarcoidosis and histoplasmosis, the evidence strongly suggests that they are instances of uncomplicated histoplasmosis. It is concluded that Histoplasma capsulatum may be an occasional cause of the clinicopathologic picture to which the name sarcoidosis has been given.

ACUTE DIFFUSE INTERSTITIAL FIBROSIS OF THE LUNGS

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ACUTE diffuse interstitial fibrosis of the lungs is a disease of unknown causation characterized by sudden onset of cough, hemoptysis, dyspnea, cyanosis, and fever. Death from cardiac failure or pulmonary asphyxia has occurred in the reported cases after an illness of a few weeks. The disease was first described by Hamman and Rich,[1] who reported four cases and identified one instance from the previous literature.[2] The detailed description of the pathologic anatomy that these authors presented established the fundamental change as diffuse proliferation of fibrous connective tissue which involved the interstitial tissue of the lungs, with subsequent alterations in the pulmonary circulation. Associated pathologic changes consisted of metaplasia of the epithelium of the bronchi and of the pulmonary alveolar lining, the formation of intra-alveolar hyaline membranes, and thickening of the pulmonary arterioles.

Since the original description by Hamman and Rich 10 additional instances of this disease have appeared in the literature.[3] These case reports have been abstracted by Rubin, Kahn, and Pecker.[3a] In none of the reported cases has the diagnosis been established before death and in most instances only after prolonged study of the microscopic sections. Sufficient material has not been obtained in this

From the Departments of Pathology, Internal Medicine, and Radiology of the St. Francis Hospital.

1. Hamman, L., and Rich, A. R.: Acute Diffuse Interstitial Fibrosis of the Lungs, Bull. Johns Hopkins Hosp. **74**:177, 1944.

2. Kneeland, Y., Jr., and Smetana, H. F.: Current Bronchopneumonia of Unusual Character and Undetermined Etiology, Bull. Johns Hopkins Hosp. **67**:229, 1940.

3. (*a*) Rubin, E. H.; Kahn, B. S., and Pecker, D.: Diffuse Interstitial Fibrosis of the Lungs, Ann. Int. Med. **36**:827, 1952. (*b*) Eder, H.; Hawn, C. V., and Thorn, G.: Report of a Case of Acute Interstitial Fibrosis of the Lungs, Bull. Johns Hopkins Hosp. **76**:163, 1945. (*c*) Potter, B. P., and Gerber, I. E.: Acute Diffuse Interstitial Fibrosis of the Lungs, Arch. Int. Med. **82**:113, 1948. (*d*) Beams, A. J., and Harmos, O.: Diffuse Progressive Interstitial Fibrosis of the Lungs, Am. J. Med. **7**:425, 1949. (*e*) Ferrari, M.; Caubarrère, N. L.; Bottinelli, M. D.; Mendilaharsu, C., and Giudice, D.: Neumofibrosis intersticial evolutiva: Una nueva entidad? Hoja tisiol. **9**:207, 1949; abstracted, Am. Rev. Tuberc. **61**:89, 1950. (*f*) Peabody, J. W.; Peabody, J. W., Jr.; Hayes, E. W., and Hayes, E. W., Jr.: Idiopathic Pulmonary Fibrosis: Its Occurrence in Identical Twin Sisters, Dis. Chest **18**:330, 1950. (*g*) Golden, A., and Tullis, I. F., Jr.: Diffuse Interstitial Fibrosis of the Lungs, Mil. Surgeon **105**:130, 1949.

case or in previous cases to attempt to identify an etiological agent or to explain adequately the pathogenesis of the disease.

We have recently encountered a case in which the clinical signs and symptoms and the roentgenologic findings were analogous to those previously described in this disease. The diagnosis was made after studies of the autopsy material. The fundamental tissue change in the lungs was a diffuse interstitial fibrosis, but several features not previously reported were present.

REPORT OF A CASE

A 55-year-old white bulk-oil-station operator entered St. Francis Hospital Dec. 12, 1951, and died Jan. 7, 1952 (Case 39408-D).

Chief Complaint.—The patient complained of shortness of breath, cough, and hemoptysis for two and a half months.

Past History.—Six to seven years prior to admission the patient had brucellosis (undulant fever) which responded to therapy. There had been no recurrence. A tonsillectomy was performed in 1941. During the past 20 years the patient had consulted several physicians because of a chronic sinus infection associated with a postnasal drip. He had been given various antibiotics, sulfonamide compounds, and antihistaminics.

Family History.—The family history was noncontributory.

Present Illness.—The patient stated that he had had muscular weakness for a year and during this time had lost 20 lb. (9.1 kg.) in weight. Ten weeks before his admission a cough developed which was most pronounced when he was in the supine position. The cough was productive of mucus streaked with bright red blood. He was admitted to another hospital Oct. 9, 1951, and discharged three days later with the diagnosis of allergic bronchitis and nasosinusitis. A chest roentgenogram taken at this time was normal except for a slight increase in density over the right leaf of the diaphragm. The blood sedimentation rate was 5 mm. in one hour. After discharge from the hospital, the patient continued to raise small amounts of clear mucus which contained blood. A month prior to admission to St. Francis Hospital, dyspnea developed and became increasingly severer, until he was able to walk only a few steps and was dyspneic at rest.

Results of Physical Examination.—The patient's temperature was 98 F.; the pulse rate was 74 per minute, and the respiration rate was 24 per minute. The blood pressure was 100/70 mm. Hg. The patient was well developed and well nourished but in obvious respiratory distress. There was slight cyanosis of the lips and nail beds. The fingers were not clubbed. There was moderate increase in the anteroposterior diameter of the chest, which was hyperresonant to percussion. Auscultation revealed a few moist rales in the base of the left lung. The heart was not enlarged to percussion, and the sounds were distant. The aortic second sound exceeded the pulmonic second sound in intensity. The liver was not enlarged, and there was no dependent edema.

Laboratory Findings.—The red blood cell count was 4,800,000 and the white blood cell count 7,450 per cubic millimeter. The differential count was 75 segmented forms, 7 band forms, 17 lymphocytes, and 1 eosinophile per 100 cells. The hemoglobin concentration was 15.9 gm. per 100 cc. The blood sedimentation rate (Wintrobe method) was 28 mm. in one hour. The fasting blood sugar was 66 mg. and the blood chlorides were 538 mg. per 100 cc. The total serum protein was 5.6 gm. per 100 cc., with 3.7 gm. of albumin and 1.9 gm. of globulin. Serum agglutination for brucellosis was negative. The Kline reaction of the blood was negative. The carbon dioxide content of the blood was 43.6 vol. %. The results of urinalysis were normal. No acid-fast organisms were seen on direct smears of the sputum. A few eosinophiles were present. Sputum culture grew nonhemolytic streptococci and Staphylococcus aureus. In vitro sensitivity tests showed inhibition with terramycin, aureomycin, chloramphenicol (chloromycetin®), and bacitracin. The electrocardiogram was considered to be a normal tracing.

Chest Roentgenogram.—There was advanced peribronchial thickening at the lung bases, especially the left. The pleura overlying the dome of the left diaphragm was thickened, and there was a small amount of fluid in the left costophrenic angle. The roentgenologic diagnosis was chronic bronchial disease with a superimposed acute pneumonitis in the left lung.

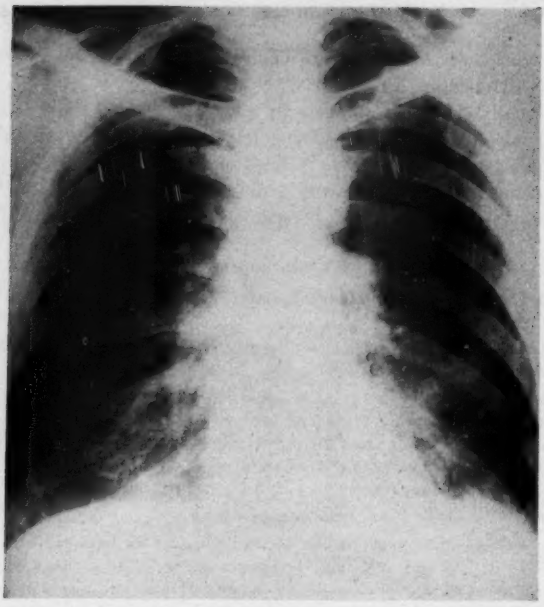

Fig. 1.—Admission roentgenogram. Note peribronchial thickening at both bases.

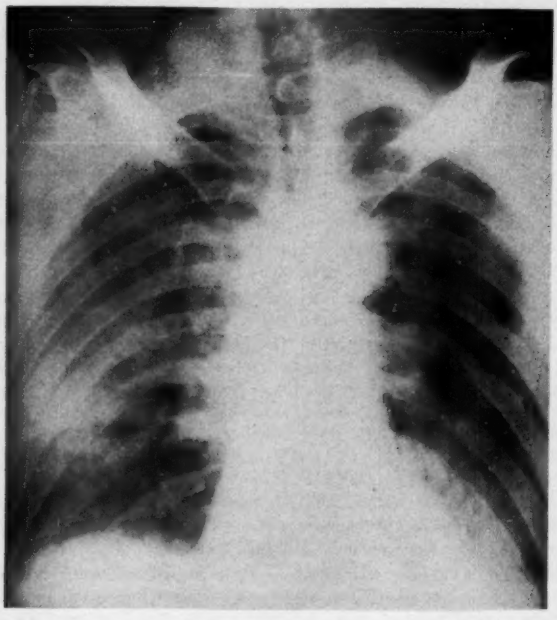

Fig. 2.—Roentgenogram made with portable apparatus on fourth hospital day. Pneumonic consolidation is plainly visible.

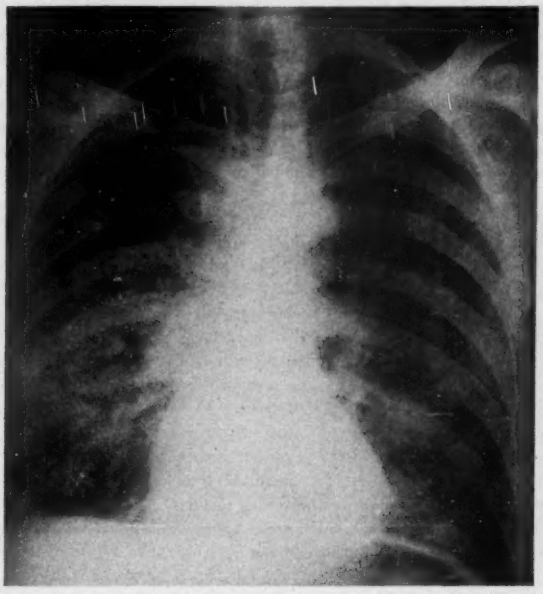

Fig. 3.—Roentgenogram taken on 17th hospital day. There are diffuse peribronchial densities.

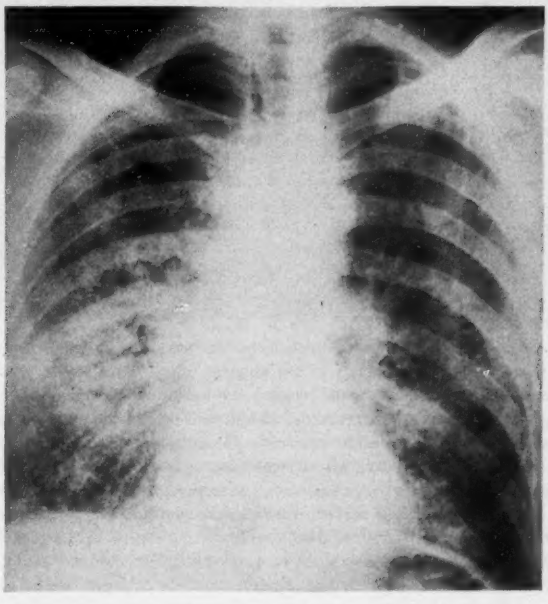

Fig. 4.—Last roentgenogram of chest, taken on 22d hospital day, five days before death.

Hospital Course.—The day following admission treatment was started with potassium iodide and cortisone (cortone®). On the fourth hospital day there was an acute episode of pleuritic pain at each base, weakness, and elevation in temperature to 100.6 F. The patient became deeply cyanotic. Faint, moist inspiratory rales were auscultated at the bases. There was no dulness to percussion. A roentgenogram of the chest made with portable apparatus showed an area of alveolar consolidation in the axillary segment of the right upper lobe. Roentgenographically, this was thought to be a bacterial pneumonia. Changes in the peribronchial structures could not be determined because of motion on the film. The patient was given penicillin, chloramphenicol, and digitoxin. Continuous oxygen administration was started with slight improvement of the dyspnea and cyanosis. An electrocardiogram taken the next day revealed lowered T waves in Leads II and III, V_4 and V_6, and aVf. On the seventh hospital day bronchoscopic aspiration was performed. Frothy, blood-tinged sputum was removed with little relief of symptoms, and continuous oxygen therapy was required. Streptomycin therapy was begun. No improvement occurred in two days; administration of both streptomycin and chloramphenicol was discontinued, and aureomycin was given. A chest roentgenogram on the 17th hospital day revealed diffuse, strand-like densities radiating outward from the hilar areas in peribronchial distribution and involving all but the apices of the lungs. These changes were more advanced in the right lung. An electrocardiogram suggested first-degree heart block, with a P-R interval of 0.24 second. The T waves were flat in Leads V_1 and aVL and inverted in Lead aVR. Corticotropin, 25 mg. daily, was given. The dose was soon increased to 25 mg. every six hours. On Jan. 4, 1952, the 22d hospital day, severer dyspnea and cyanosis developed. The chest remained hyperresonant and free from rales. The sputum still contained streaks of blood. There was no fever. The total serum protein was now 3.6 gm. per 100 cc., with 2.4 gm. of albumin and 1.2 gm. of globulin. The dyspnea and cyanosis gradually became severer, and the patient died Jan. 7, 1952, the 27th hospital day.

Report of Autopsy.—Gross Examination: The lungs completely filled the thoracic cavity and partially overlapped the mediastinal structures anteriorly. The visceral and parietal layers of the pleura were smooth and glistening, except for fibrous adhesions over the posterior surface of the right lung. There was anthracotic accentuation of the lobular markings. There was no free fluid within the pleural cavities. The lungs were heavy and weighed 2,625 gm. The consistency was firm, and there was consolidation of both lungs, which was most advanced in the lower lobes and in the right upper lobe. The cut surface of each lung was dry, gray, and finely granular and contained multiple grayish-yellow foci 1 to 3 mm. in diameter. No fluid or purulent material was expressed from the cut surface. The mucosa of the trachea and major bronchi was slightly reddened, and there was no exudate within the lumina. There was an ulcer which measured 0.5 cm. in diameter in the mucosa of the secondary branch of the right lower lobe bronchus. Beneath this ulcer there was a nodule of firm, grayish-white, finely granular material that measured 1 cm. in diameter. The intimal surface of the pulmonary arteries was smooth except for that of the primary branches, which contained a few elevated, grayish-yellow plaques. There were numerous lymph nodes 1 to 2 cm. in diameter at the hilus of each lung and about the tracheal bifurcation. On cut section they were soft and heavily stippled with anthracotic pigment.

The heart weighed 350 gm. There was slight fibrous thickening of the leaflets of the mitral valve, with fusion and shortening of the chordae tendineae. The tips of the papillary muscles contained fibrous plaques. There was a thick, cord-like tendinous insertion from the anterior papillary muscle to the posterior surface of the anterior mitral valve leaflet. Thickening of the cusps of the aortic valve and adhesions between the cusps at the commissures were noted. The myocardium of the left ventricle measured 15 mm. and that of the right 4 mm. in thickness. The tricuspid valve was 135 mm. in circumference, the pulmonic, 75 mm., the mitral, 100 mm., and the aortic, 75 mm. The coronary arteries contained a few arteriosclerotic plaques, but the lumina were patent. No gross pathologic changes were present in the myocardium.

The liver weighed 1,870 gm. The surface was discolored yellow. The parenchyma bulged from the cut surface, and there was dark red discoloration in the region of the central veins. Gross examination of the gall bladder and bile ducts, pancreas, spleen, adrenal glands, kidneys, and ureters showed no specific pathologic change. The prostate was enlarged, and on cut section there were numerous well-circumscribed nodules containing small cystic spaces. The esophagus and

stomach were normal. On the posterior wall of the first part of the duodenum there was an ulcer 6 mm. in diameter. On cut section disruption of the muscle fibers and replacement with scar tissue were noted. There were petechiae in the mucosa of the large intestine and edema of the submucosa.

Microscopic Examination.—Multiple microscopic sections taken from all lobes of the lungs revealed essentially the same pathologic change. There was widening of the alveolar septa by multiplication of fibroblasts with large stellate nuclei and long cytoplasmic processes. Capillary

Fig. 5.—Gross appearance of lung. Cut surface is dry and granular, and alveoli are distended.

dilatation and edema further enhanced the widening. There was frequent rupture of the septal capillaries. A few macrophages filled with granular brown pigment were also present. There were also occasional giant cells containing 5 to 20 nuclei of uniform size and shape. The alveolar lining cells were swollen, and near the periphery of the lungs there was slight metaplasia.

The alveolar spaces were dilated, with frequent rupture of the septa. They were filled with eosinophilic fibrin with a characteristic arrangement. In the central portion of the alveolus the fibrin was finely granular and deeply eosinophilic. Peripheral to this it was arranged in delicate strands forming a lace-like pattern. In a few alveoli there were occasional polymorphonuclear leucocytes.

The capillaries throughout the lungs were dilated, and there was hyperplasia of the endothelium. Arterioles and arteries were also dilated, and there was fibrous thickening of the adventitia. There was no cellular exudate within the terminal bronchioles, and there was no dilatation.

These pathologic changes varied somewhat in progression throughout the lungs. Near the bases, the fibrous tissue within the septa was more mature and edema was minimal. There was also slight transmigration of fibroblasts into the periphery of the alveolus, representing an attempt at organization. Toward the apices of the lungs, the fibroblasts were immature and edema was more extensive.

Within the nodule adjacent to the right lower lobe bronchus there were atypical epithelial cells which were arranged in nests and cords. There was no tendency to form glands or line

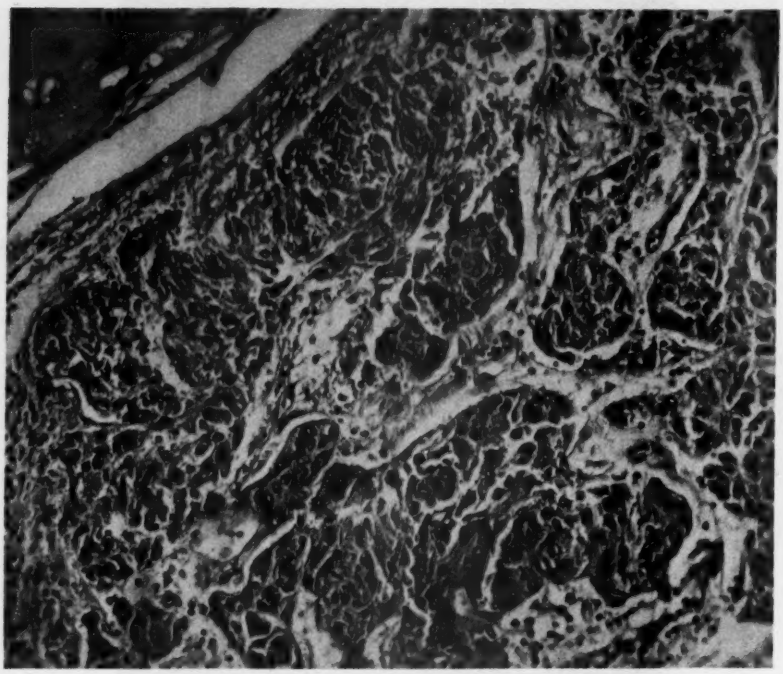

Fig. 6.—Undifferentiated bronchogenic carcinoma. Entire tumor measured 1 cm. in diameter, and there was no extension from site of origin. Hematoxylin and eosin stain; × 175.

spaces. Within the cell groups zones of necrosis and hemorrhage were present. Multiple sections of the hilar and tracheal lymph nodes failed to reveal evidence of metastases.

Microscopic examination of the heart disclosed occasional dilatation and blunting of the nuclei of the cardiac fibers. There was no cellular infiltration. The central veins and adjacent sinusoids within the liver were dilated. The hepatic cells adjacent to the central veins were necrotic. Within the peripheral cells there were small and large fat droplets. The prostate contained numerous nodules composed of epithelial acini. Within one nodule there was squamous metaplasia of the cells lining the acini. No evidence of malignant transformation was seen. The ulcer of the duodenal mucosa extended partially into the muscularis. There were deposition of fibrous connective tissue in the muscularis and infiltration with lymphocytes.

Anatomical Diagnoses.—The anatomical diagnoses were acute interstitial fibrosis of the lungs; fibrin within the pulmonary alveoli; fatty metamorphosis of the liver; chronic passive congestion of the liver; undifferentiated carcinoma of the right lower lobe bronchus; chronic peptic ulcer of the duodenum; nodular hyperplasia of the prostate, and healed infarct of the prostate.

PATHOLOGIC ANATOMY

The gross pathologic changes observed in the lungs differed in some respects from those in the previously reported instances of diffuse interstitial fibrosis. These changes were undoubtedly the result of the presence of large amounts of fibrous

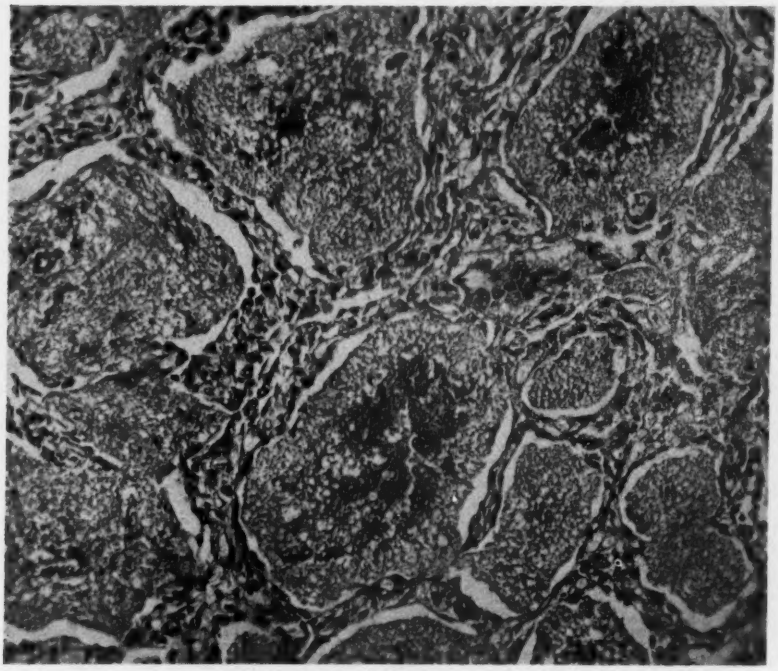

Fig. 7.—Representative section of lungs. Thickening of alveolar septa and proliferation of young fibroblasts in interstitium are prominent. Fibrinous material in alveolar spaces is devoid of cellular infiltration. Hematoxylin and eosin stain; × 175.

connective tissue throughout the parenchyma and deposition of fibrin within the alveoli. The consistency of the lungs was firm, with almost complete consolidation of all lobes. A conspicuous finding was the complete absence of fluid in the pleural cavities and the lack of any exudate on the surfaces of the visceral pleura. The cut surface was gray and contained multiple small yellow foci which were erroneously interpreted as representing granulomata. The cut surface was dry and finely granular, and no exudate could be identified with pressure. The mucosa of the trachea and bronchi was slightly reddened, but no exudate was present in the lumina.

The microscopic appearance was equally puzzling and presented an entirely new picture to us. There were two conspicuous processes. The first consisted of a diffuse increase in interstitial connective tissue with proliferation of an abundance of young fibroblasts within the alveolar septa and in the perivascular and peribronchial regions. A proliferation of young capillaries in the interalveolar regions was associated with interstitial edema, and the resulting separation of cellular constituents produced a widening of the alveolar septa. In the peripheral portions of the lower lobes of the lungs there was disruption in the continuity of many of the alveolar septa, with resultant formation of large coalescent emphysematous spaces. The

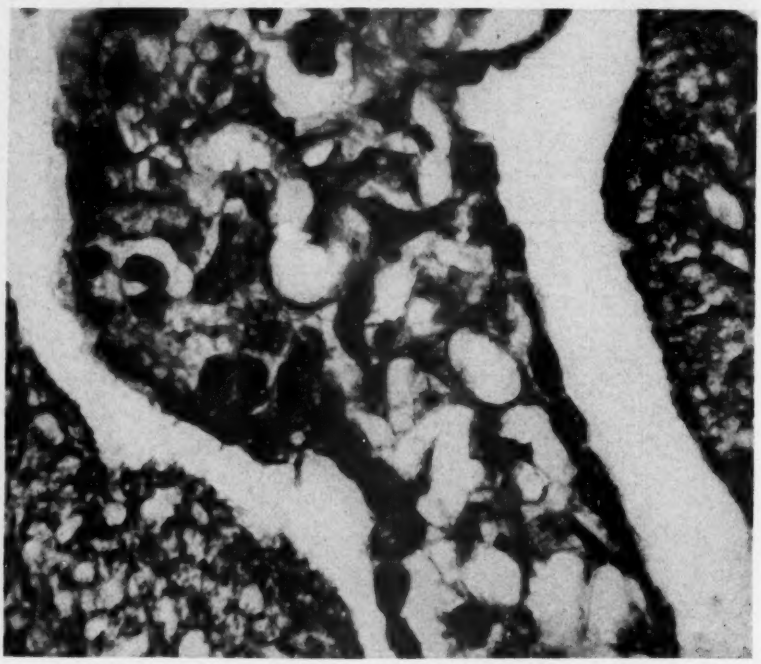

Fig. 8.—High magnification of thickened alveolar septum. Enlargement of pulmonary alveolar lining cells, interstitial edema, and fibroblastic proliferation can be seen. There is complete absence of infiltration with inflammatory cells. Hematoxylin and eosin stain; × 480.

ruptured fragments of the septa were thickened three to four times the normal diameter and contained many young fibroblasts with large stellate nuclei. Cytoplasmic processes formed a thick interlacing network that surrounded deposits of granular, non-nucleated, eosinophilic material which contained a fine network of fibrinous material.

The second conspicuous reaction was filling of the alveolar spaces with a fibrinous material which had a distinct arrangement. The fibrin in the central portion of the alveoli was deeply eosinophilic and finely granular without a reticular structure. Radiating from this central zone were stellate fibrinous strands forming

an interlacing pattern. There was no cellular infiltration of an inflammatory type either in the alveolar transudate or within the interstitial tissue. In contrast to the observation of Hamman and Rich [1] and also Beams and Harmos,[3d] metaplasia of the pulmonary alveolar lining or of the terminal bronchiolar epithelium was an inconspicuous part of the microscopic pattern in our case. The metaplasia in this instance was observed only at the periphery of the lungs and was confined to the pulmonary alveolar lining. However, in this portion of the lung the fibrin deposits

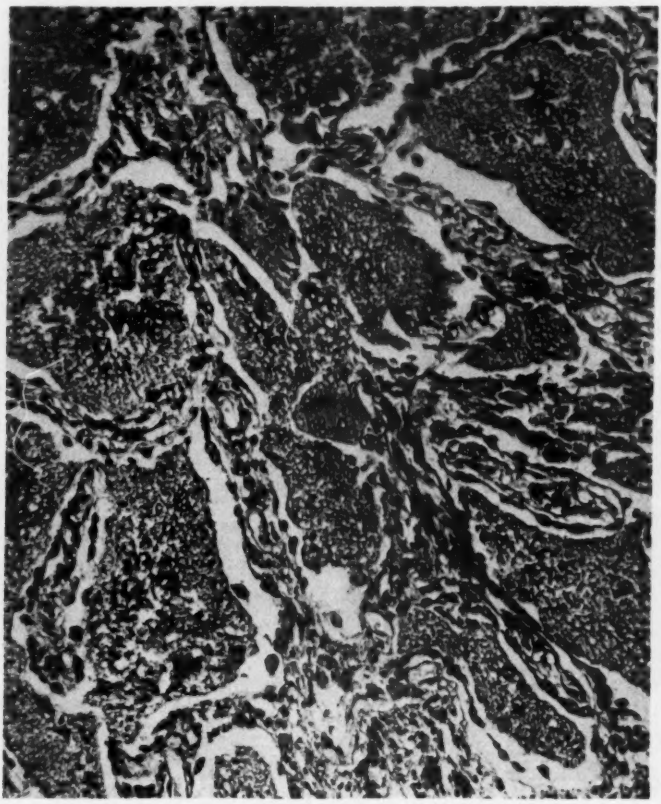

Fig. 9.—Giant cell within pulmonary alveolus. Early metaplasia of pulmonary alveolar lining is present, although rarely found except at periphery. Hematoxylin and eosin stain; × 175.

in the alveoli were not extensive. A migration of young fibroblasts into the fibrinous material in the alveoli was considered indicative of an early attempt at organization. The transposition of fibroblasts was salient at the periphery of the alveolar spaces and adjacent to the ruptured ends of many of the alveolar septa.

The capillaries within the lung were dilated, and there was hyperplasia of the lining endothelium. The larger vascular channels also had distended lumina, and there was multiplication of the surrounding connective tissue. In a few sections

multinucleated cells were present which apparently had no phagocytic activity. It is most probable that they represented symplasmatic giant cells formed by fusion of adjacent fibroblasts and were not related to the presence of an irritating substance. Careful search failed to demonstrate the presence of eosinophilic leucocytes or any other cellular elements of the blood within the bronchi, alveoli, or interstitial tissue. Eosinophiles were described in large numbers in the cases of Hamman and Rich [1] and may be a prominent part of the microscopic pattern, but the prolonged admin-

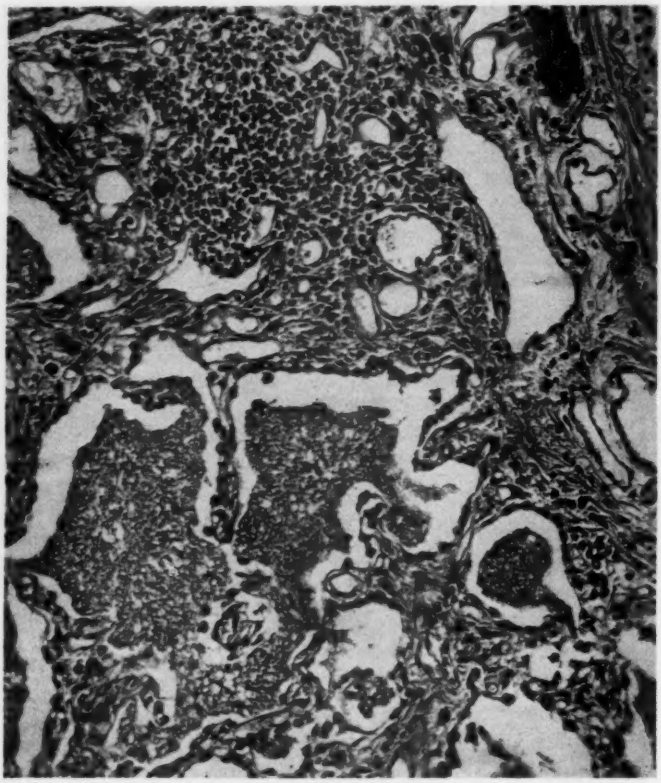

Fig. 10.—Section of peripheral portion of lung beneath pleura. Emphysema and alveolar lining metaplasia occurred more frequently in this portion of lungs. Amount of interstitial fibrosis was less in subpleural regions. Hematoxylin and eosin stain; × 175.

istration of large doses of cortisone in our case could have eliminated these cells from the circulating blood.

No stainable bacteria were demonstrated in the fibrin within the alveoli, the interstitial tissue of the lungs, or the bronchi by either the Gram-Goodpasture or the Brown technique. Smears of the fresh cut surface of the lungs contained no bacteria, and cultures were sterile.

The undifferentiated carcinoma present within the secondary branch of the right lower lobe bronchus was small, measuring 1 cm. in diameter. The ulcer on the surface of the bronchial mucosa was 5 mm. in diameter, and the bulk of the tumor was present between the bronchial cartilages. There was no stenosis of the lumen of the bronchus. Examination of many sections taken from all lobes of the lungs and the bronchopulmonary and tracheobronchial lymph nodes has failed to show any extension of the tumor beyond the primary site of origin.

CLINICAL MANIFESTATIONS

The clinical findings in the previously reported cases were abstracted by Rubin, Kahn, and Pecker.[3a] The commonest signs and symptoms encountered in these instances and in the case reported in this paper are summarized in the Table. There appears to be no predominance of the disease in racial, sexual, or age groups. Nine of the patients were female, and seven were male. Four patients were Negroes, and 12 belonged to the white race. The ages of the patients ranged from 21 to 68 years, and the average age was 44 years. The occupation of the patients apparently is not important and does not imply contact with any specific toxic agent. Our patient was employed in the sale of bulk gasoline, which brought him into contact with fumes of benzine products, but no similar employment or association was present in previous instances of the disease. Of the patients previously reported on, one was employed as a welder [3g] and one as a printer.[3c] The occupations of the other patients were sedentary without obvious contact with noxious agents.

In six of the previous cases there was a prodromal period with incipient symptoms of nonproductive cough, weakness, and weight loss extending from six months to seven years.[4] The clinical studies during this period, although incomplete, demonstrated no clinical signs or roentgenographic evidence of pulmonary disease. The history in all instances failed to indicate the presence of a preexisting pulmonary disease which might have led to pulmonary fibrosis of any type. No instances of preceding viral or atypical pneumonitis were encountered. There is no evidence that can incriminate a bacterial agent as a possible etiological factor. Fever was present in only one case during the prodromal period,[1] and in the majority of instances this symptom was absent during the entire course of the disease, especially in all cases with antibiotic therapy. Leucocytosis occurred sporadically throughout the course of the disease but was not a persistent finding and was usually terminal. Our patient's sputum remained free of purulent material, a finding which indicates the absence of suppurative inflammation in the tracheobronchial tree. These findings suggest that the pathogenesis of the disease is not on the basis of a previous or concomitant bacterial infection.

During the active phase of the disease, there is a rapid progression of symptoms of increasingly severe dyspnea, with cyanosis and occasional hemoptysis. The roentgenograms during this period show a progressive dissemination of peribronchial densities which usually begins in the lower lobes and extends superiorly to involve the entire lungs. In six reported cases there were hypertrophy and dilatation of

4. (*a*) Hamman and Rich.[1] (*b*) Footnote 3*c* to *g*. (*c*) Peabody, H. D., Jr.; Moersch, H. J., and Edwards, J. E.: Clinically Indeterminate Pulmonary Fibrosis: A Pathologic Study, J. Thoracic Surg. **21**:519, 1951.

*Summary of Data on Cases of Acute Interstitial Fibrosis of the Lungs**

Author	Age	Sex	Race	Occupation	Location	Presenting Symptoms	Duration of Disease	Heart Size	Clinical Chest Findings	WBC
Hamman & Rich [1]	47	M	N	Laborer	Md.	Cough, dyspnea, edema	4 mo.	Slight enlargement; rt. ventricle, 10 mm.	Harsh breath sounds, moist rales	10,700
Hamman & Rich [1]	21	F	N	N. S.	Md.	Dyspnea, fever	46 days	350 gm.; rt. ventricle, 6 mm., dilated	Limited excursions, focal dulness, moist rales	15,000
Hamman & Rich [1]	37	F	N	N. S.	Md.	Hemoptysis (pulmonary tuberculosis)	31 days	Dilatation and hypertrophy of rt. ventricle	Few fine rales	17,800
Hamman & Rich [1]	68	F	W	N. S.	Md.	Cough, dyspnea	6 mo.	345 gm.; slight dilatation of lt. atrium	Crepitant rales, dulness at bases	55,000
Eder & others [2b]	47	M	W	Waiter	Mass.	Slight dyspnea, weight loss	4 mo.	310 gm.; rt. ventricle, 7 mm.	Few rales in right axilla	25,000
Potter & Gerber [2c]	38	M	W	Printer	N. J.	Fatigue, weight loss	32 wk.	300 gm.; dilatation and hypertrophy of rt. ventricle	Scattered rales	82,000
Beams & Harmos [2d]	32	M	W	Seaman	Ohio	Dyspnea	15 mo.	470 gm.; rt. ventricle, 6 mm., dilated	Basilar rales	16,000
Peabody & others [2f]	44	F	W	Housewife	N. S.	Cough, hemoptysis, dyspnea	3½ yr.	Normal	None	N. S.
Ferrari & others [2e]	63	F	W	N. S.	Uruguay	Cough, dyspnea	4 mo.	N. S.	N. S.	N. S.
Ferrari & others [2e]	41	M	W	N. S.	Uruguay	Weight loss, cough	1 yr.	N. S.	N. S.	N. S.
Peabody & others [4c]	47	F	W	N. S.	Minn.	Dyspnea	2 yr.	N. S.	N. S.	N. S.
Golden & Tullis [2g]	41	M	W	Welder	Tenn.	Cough, dyspnea, edema	6 to 7 yr.	Hypertrophy and dilatation of rt. ventricle	Wheezing rales	N. S.
Golden & Tullis [2g]	42	F	N	N. S.	Tenn.	Weakness, dyspnea, edema	.....	Hypertrophy and dilatation of rt. ventricle	Rales	N. S.
Rubin & others [2a]	44	F	W	Secretary	N. Y.	Dyspnea, edema	2½ yr.	245 gm.; dilated pulmonary conus and lt. atrium	Wheezing rales	8,400
Kneeland & Smetana [3]	47	F	W	N. S.	N. Y.	Fever, hemoptysis, urticaria	10 wk.	400 gm.; moderate hypertrophy of rt. ventricle	Slight dulness, rales, bronchial sounds	N. S.
Callahan & others	55	M	W	Bulk-oil-station operator	Kan.	Cough, dyspnea, hemoptysis (bronchovenic carcinoma)	3½ mo.	350 gm.; rt. ventricle, 4 mm.	Hyperresonance, moist basilar rales	14,100

* N. S. means not stated.

the heart and thickening of the myocardium of the right ventricle.[5] In our case, there was no clinical evidence of cardiac hypertrophy and no enlargement of the heart was observed at autopsy. However, the extensive deposition of fibrinous material within the alveoli without anatomical evidence of an exudative process indicates terminal pulmonary edema and transudation of a fluid high in protein content into the alveolar spaces. It is interesting that during this period the serum protein fell precipitously, and the only adequate explanation of this loss from the plasma is the accumulation of protein in the alveolar spaces. The roentgenologic findings in this case are similar to those in previously reported instances, consisting of peribronchial thickening beginning at the bases of the lungs and extending to all lobes in the short period of two weeks. The rapid progressive increase in density to involve all lobes of the lungs was undoubtedly a result of interstitial fibrosis but may partly be a result of the accumulation of fibrinous material in the alveolar spaces. The rapid progression of the lesions would be unusual in any but an inflammatory disease. The peribronchial spread of primary or metastatic tumors, Hodgkin's disease, lymphosarcoma, and sarcoidosis does not develop so rapidly. The roentgenographic findings associated with the symptoms of cough, hemoptysis, and dyspnea with progressing cyanosis are sufficient to establish a clinical diagnosis.

CAUSATION AND PATHOGENESIS

The anatomical findings in this case, as in those previously reported, imply a single pathologic process with the production of a variety of physiological disturbances. The fundamental pathologic change is an increase in the interstitial connective tissue with impairment of the blood flow through the lungs. The subsequent development of interstitial edema and transudation of fluid into the alveoli interferes with the gaseous exchange and is responsible for the clinical symptoms of dyspnea and cyanosis. Peripheral emphysema results in uninvolved portions from hyperpnea and excessive respiratory efforts by the patient.

The complete lack of cellular infiltration implies that the basic lesion of interstitial fibrosis is not bacterial and if inflammatory must be a result of virus infection or chemical irritation. The absence of a definite history of exposure is against the latter but does not exclude it. The possibility of a viral agent must be seriously considered. However, adequate studies are not available because of the inability to establish a clinical diagnosis or because of the inability of the pathologist to recognize the disease from the gross appearance and obtain suitable material. In some respects the changes in the lungs are similar to lymphedema seen in other tissues. The production of fibroblasts is the most frequent response to the presence of lymph in the intercellular spaces but is usually associated with chronic obstruction to lymph flow. Drinker, Field, and Homans[6] described similar pathologic changes in the subcutaneous tissues of dogs following repeated injection of a suspension of silica in a solution of quinine into the regional lymphatic channels. Such a sequence of events may be a factor in the development of the changes in the interstitial tissues of the lung. There is no evidence that such a process has occurred, but a distinct

5. Hamman and Rich.[1] Kneeland and Smetana.[2] Footnote 3*c* and *g*.

6. Drinker, C. K.; Field, M. E., and Homans, J.: The Experimental Production of Edema and Elephantiasis as a Result of Lymphatic Obstruction, Am. J. Physiol. **108**:509-520, 1934.

similarity exists in the anatomical changes. This type of pathologic change could explain the prodromal stage and the acute phase of the disease. The causation, however, remains obscure, and further studies are needed.

Peabody and associates [31] reported the occurrence of a pulmonary disease with roentgenologic and clinical findings similar to those in a proved fatal case of diffuse pulmonary fibrosis in the patient's twin sister. Information concerning the subsequent developments in this instance has not been reported.

SUMMARY

A case of diffuse interstitial fibrosis of the lungs is reported. The clinical and roentgenographic observations in this case are described, and the clinical data on the previously reported cases are tabulated. The pathologic changes in the lungs are similar in many respects to those in cases previously reported, except for the accumulation of large amounts of fibrinous material in the alveolar spaces. The pathogenesis and causation are discussed on the basis of the clinical findings and the pathologic changes observed in the autopsy specimens. The possibilities of a viral agent or of chronic pulmonary lymphedema as an etiological factor are presented.

PORPHYRIA CUTANEA TARDA

Investigation of a Case, Including Isolation of Some Hitherto Undescribed Porphyrins

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WALDENSTRÖM [1] has divided the porphyrias into the following groups:

1. Porphyria congenita, a familial disease, probably recessive in character, present at birth and characterized by sensitivity to light, pigmented bones and teeth, and greatly increased coproporphyrin and uroporphyrin excretion, mainly of Series I.

2. Porphyria cutanea tarda, a disease in which the porphyrinuria and sensitivity to light develop later in life and a sclerodermic tendency is noticeable. The occurrence of colic and the occasional excretion in the urine of uroporphyrin suggest a relationship with the group next to be described. Severe nervous symptoms have not yet been reported.[2]

3. Porphyria acuta, a familial disease, dominant in character, accompanied with the excretion of coproporphyrin and uroporphyrin mainly of Series III. The following clinical forms have been recognized:

(*a*) Latent porphyria in which there is increased uroporphyrin excretion without any clinical manifestations of porphyria; such patients as have been encountered have always been relatives of patients with overt forms of the disease (see *b*, *c*, *d*, and *e*)

(*b*) The pure abdominal form with abdominal symptoms but no paralyses

(*c*) The pure nervous form with paralyses but no abdominal symptoms

(*d*) Classical acute porphyria with colics and paralyses

(*e*) The comatose form in which the coma of the terminal stage predominates

Waldenström found the excretion of porphobilinogen, thought to be a dipyrrylmethene porphyrin precursor, to be a characteristic feature of porphyria acuta. He considered porphyria cutanea tarda to be a distinct disease entity, although the

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1. Waldenström, J.: Acta med. scandinav., Supp. 82, p. 1, 1937.

2. Nervous symptoms were absent in the case described in this paper; however, one of us (C. R.) has seen undoubted cases of porphyria cutanea tarda in which nervous symptoms have been present.

disease in patients exhibiting these characteristics has by others been referred to as of "mixed" type.[3] It is probably identical with the "chronic porphyria" of Günther's classification. Cases are rare, and there is no clear evidence to suggest a familial incidence. In none of the cases mentioned by Waldenström did he detect porphobilinogen in the urine.

In 1948 Gray, Rimington, and Thomson [4] reported their investigation of a case of porphyria corresponding closely to porphyria cutanea tarda. The patient was a young man in whom colic and sensitivity to light developed in early manhood. He became deeply jaundiced after each attack of colic, and porphyrin could be demonstrated in his blood plasma at those times. There were never any nervous symptoms, and the patient appeared to improve gradually in health during the six years he was under observation. This improvement has been maintained since. The striking observation was made in his case that there was a reciprocal relationship between the amount of porphyrin excreted in the urine and the amount excreted in the feces. During attacks, urinary porphyrin rose and fecal porphyrin fell, while in remission periods his urine was practically free from porphyrin but his feces were loaded with pigment.

Investigations of liver function left little doubt that the jaundice was hepatogenous, but it seemed unlikely that the porphyrinuria associated with each attack was merely the result of an episode of hepatic dysfunction. Gray, Rimington, and Thomson attempted to isolate the porphyrins excreted by this patient but were unsuccessful, apart from the recognition of some coproporphyrin I. The ether-insoluble porphyrin which made up the greater part of the urinary porphyrin would not yield a crystalline methyl ester, and attempts at decarboxylation indicated that it was of an unusual type showing great instability. Hesitation was felt, therefore, in making a precise diagnosis in this case.

Some time ago a case very similar to that just described came under the observation of one of us at the Royal Infirmary, Sheffield. As the technical methods of separating, characterizing, and determining porphyrins had been very greatly improved since 1947,[5] it was decided that a thorough investigation of this case should be made jointly. Although the patient died in March, 1950, many clinical and biochemical data have been accumulated and are presented in this paper. Our observations include the isolation of four hitherto unknown porphyrins and the finding of a close relationship between urinary uroporphyrin excretion and arterial blood pressure in this patient. The association between attacks of colic with jaundice and the diversion of porphyrin from the fecal to the urinary route of excretion was similar to that found by Gray, Rimington, and Thomson [4] in their case. It is of interest that the patient showed some signs of adrenal cortical insufficiency.

As a result of a study of the clinical manifestations in this new case, and because of the chemical investigations which it was possible to make, we feel justified in regarding it as a typical and substantiated case of porphyria cutanea tarda.

3. Calvy, G. L.: Surg., Gynec. & Obst. **90**:716, 1950.

4. Gray, C. H.; Rimington, C., and Thomson, S.: Quart. J. Med. **17**:123, 1948.

5. (*a*) Nicholas, R. E. H.: Biochem. J. **48**:309, 1951. (*b*) Nicholas, R. E. H., and Rimington, C.: Scandinav. J. Clin. & Lab. Invest. **1**:12, 1949; (*c*) Biochem. J. **48**:306, 1951. (*d*) Sveinsson, S. L.; Rimington, C., and Barnes, H. D.: Scandinav. J. Clin. & Lab. Invest. **1**:12, 1949. Rimington, C., and Sveinnson, S. L.: ibid. **2**:209, 1950.

REPORT OF CASE

Clinical History.—The patient was a boy 14 years of age when first seen in August, 1948. He was an only child of British parents, resident in Derbyshire, all his relatives and recent ancestors coming from the same area. The parents were not known to be related. He was always healthy and well until 1945, apart from the common childhood exanthemata; both parents and schoolteachers had, however, noted that he appeared to be mentally rather backward, and he had not made particularly satisfactory progress at school.

In May, 1945, for the first time, he had a septic skin eruption which was regarded as impetigo by his family physician, and this cleared after a few weeks. He was well in all respects until the spring of 1946, when once again he had "septic blisters" on his hands and face for about six weeks.

In July, 1947, several crops of blisters developed on the face and on the backs of his hands. These were itchy and burst and scabbed over, but took some time to clear with local penicillin applications and left residual scars. At this time he had not noticed any urinary abnormality, nor did he have any accompanying symptoms of pain or colic.

In November, 1947, he had an episode of diarrhea and vomiting, regarded as infective in nature and treated with sulfonamides. This lasted three weeks and was not noticed to be associated with any urinary abnormality, nor was there any accompanying abdominal pain. He was well during the rest of that winter but on "a few occasions" noted that his urine appeared very dark; in March, 1948, the blisters recurred, and he was seen in the Sheffield Royal Hospital, where the skin lesions were diagnosed as hydroa aestivale by Dr. I. B. Sneddon. At this stage his urine was examined for porphyrin, which could not be detected on spectroscopic examination, and Ehrlich's aldehyde test for porphobilinogen was negative.[6] The skin lesions cleared satisfactorily but recurred in August after a trip to the seaside, and he was admitted to Sheffield Royal Infirmary under the care of Dr. Sneddon and subsequently transferred to the care of Prof. E. J. Wayne when porphyrins were detected in the urine on spectroscopic examination.

First Admission to Hospital, September, 1948.—While in hospital he had a severe attack of epigastric pain and pain in his loins, and he became definitely jaundiced. His clinical course from this time until his death 18 months later is summarized in Figure 1.

On examination he was seen to be a thin, malnourished boy, weighing 95 lb. (43.1 kg.). His face and hands were covered with fresh and old lesions of hydroa aestivale with considerable crusting and scabbing (Figs. 2 and 3). His skin was slightly pigmented in the exposed areas, and his conjunctivae were definitely icteric. His nails were brittle and deformed with transverse scarring, and all were pigmented in their central portions. His teeth were sound and normal in color. His heart and lungs were normal, the pulse rate being consistently about 70 per minute and the blood pressure 130/90. A full and detailed neurological examination gave normal results in all respects. There was no apparent abdominal abnormality.

After his first few days in hospital, he had no further attacks of abdominal pain, and his jaundice progressively diminished. The only therapy given was penicillin parenterally, and with this his skin lesions dried up, and he was discharged on Nov. 20, 1948, with no active lesions, no jaundice, and a gain in weight of 7 lb. (3.2 kg.). He was afebrile and had a normal pulse rate and blood pressure throughout the whole period. Special investigations carried out during this period showed that, whereas his serum bilirubin was 8.6 mg. per 100 cc. and his serum alkaline phosphatase 26 units (King-Armstrong) on Sept. 2, 1948, his bilirubin was less than 0.2 mg. per 100 cc. and his alkaline phosphatase was 15 units on Nov. 18, 1948, with intervening intermediate results (Fig. 1). Initially there was bile in his urine, and he had positive direct and indirect van den Bergh reactions, but these were normal in a few weeks. Results of a galactose-tolerance test and a hippuric acid-excretion test were normal at a time that his serum bilirubin was 7 mg. per 100 cc. His urine contained a trace of albumin, porphyrins were present on spectroscopic examination, and the urine darkened considerably on standing until it became a dark sherry color. It fluoresced in ultraviolet light, and an amyl alcohol extract fluoresced bright pink, as did his serum and the contents of cutaneous bullae. His Wassermann reaction was negative; serum cholesterol was 240 mg. per 100 cc., and his serum electrolytes were normal,

6. Watson, C. J., and Schwartz, S.: Proc. Soc. Exper. Biol. & Med. **47**:393, 1941.

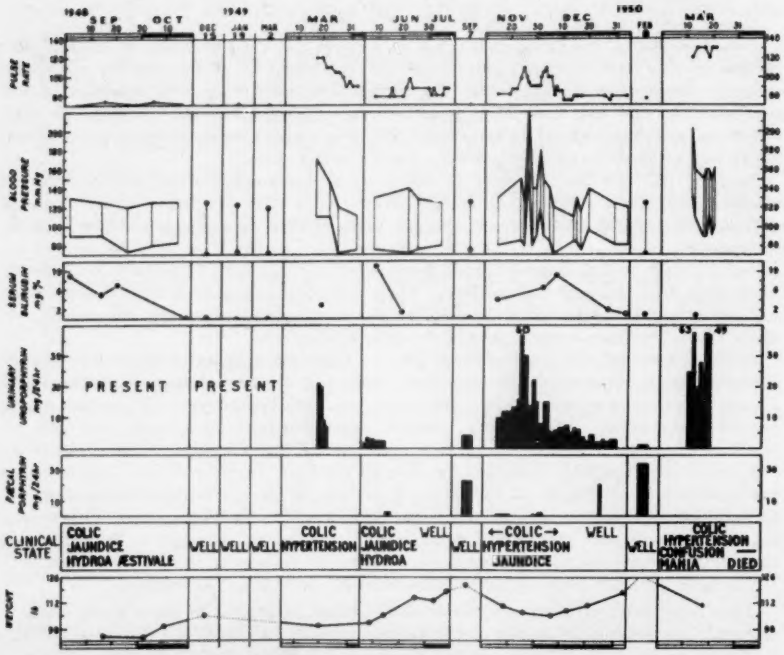

Fig. 1.—Chart illustrating clinical and biochemical features in a case of porphyria cutanea tarda for the 19 months preceding death.

Fig. 2.—Facies of a patient with hydroa aestivale accompanying porphyria cutanea tarda.

sodium measuring 355 mg., potassium, 20 mg., and plasma chlorides (as sodium chloride), 550 mg. per 100 cc. His blood urea was 38 mg. per 100 cc., and renal function was normal as judged by normal results on urine concentration and dilution and urea clearance tests. A Kepler test gave a normal result, "ratio A" being 52 (Table 1). His hemoglobin concentration was 90%; erythrocytes were 4,500,000 and leucocytes, 6,000 per cubic millimeter, with a normal differential count. Reticulocytes were less than 1% and thrombocytes (platelets) 140,000 per cubic millimeter. A sternal marrow biopsy specimen showed a normal normoblastic marrow. No cold agglutinins were detectable in his serum. A lumbar puncture was normal in all respects. Roentgenograms of chest and abdomen were normal, as was his electrocardiogram.

The absorption bands of porphyrins could be detected spectroscopically in his urine throughout this period, but at no stage was any porphobilinogen detectable. Quantitative porphyrin estimations were not made at this stage.

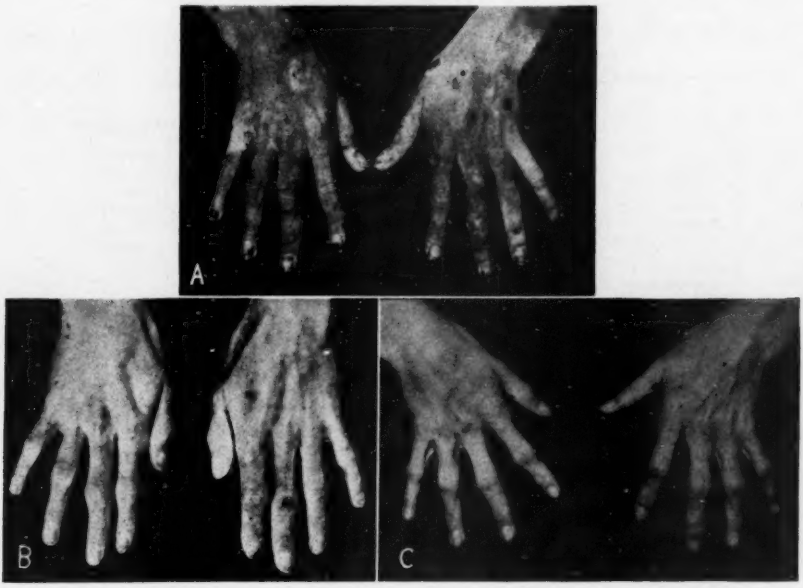

Fig. 3.—Hands of patient on (*A*) Sept. 3, 1948, (*B*) June 16, 1949, and (*C*) Dec. 3, 1949, showing lesions of hydroa aestivale and progressive development of an arthritis of rheumatoid type.

TABLE 1.—*Results of Kepler Tests (Volume of Urine Excreted, Cc.)*

Specimen	10/25/48	6/10/49	6/21/49	11/15/49	12/7/49
Night	260	455	570	210	520
1	170	76	61	88	230
2	370	159	290	221	170
3	70	59	306	62	72
4	45	50	176	38	51
Ratio A	52	13	14	32	16

After his discharge, he remained well for the next four months and had no colic or skin lesions. He was seen as an outpatient on Dec. 15, 1948, Jan. 19 and March 2, 1949, and on each occasion he was clinically well and normotensive and had no evidence of jaundice, his serum bilirubin being below 0.2 mg. per 100 cc. on each occasion. His urine, however, continued to

contain small quantities of porphyrin; amyl alcohol urine extracts showed mild pink fluorescence, and his serum fluoresced on each occasion.

Second Admission to Hospital, March, 1949.—On March 18, 1949, he was readmitted to the hospital after a five-day bout of abdominal pain and vomiting, during which his urine had been very dark. On admission he was obviously suffering considerable pain, which was adequately controlled with meperidine (pethidine), and he had lost 7 lb. in weight since the previous record in January. There was a suggestion of icterus, his serum bilirubin being 3 mg. per 100 cc.; he showed a slight tachycardia, and his blood pressure on admission was 170/110. Results of neurological examination were normal, but there was marked retinal arterial narrowing during this phase. The development of mild gynecomastia was noted. During the next two weeks his symptoms rapidly resolved, and his blood pressure on March 29, 1949, was 110/75, and there was no clinical icterus. He was discharged home on March 31, 1949, clinically well, although his urine continued to contain porphyrin. While he was in the hospital, his urine at no stage contained

TABLE 2.—*Analysis of Urines and Stools Passed During an Attack (March 18 to 23, 1949) and Subsequent Recovery (June, 1949)*

Date	Uroporphyrin, Mg./L.	Copro-porphyrin, Mg./L.	Paper Chromatographic Examination	
			Porphyrin	Amount
Urines				
3/19-20	4.22	0.214	Octacarboxylic	Ca. 50%
3/20-21	5.44	0.213	Heptacarboxylic	Trace
3/21-22	11.77	0.866	Pentacarboxylic	Ca. 25%
3/22-23	12.09	0.992	Tetracarboxylic	Ca. 25%
			Tricarboxylic	Present
			Dicarboxylic	Trace
5/31-6/2	1.74	0.104		
6/2-4	2.08	0.054		
6/4-6	1.41	0.036		
6/7-8	2.65	0.089		
6/9-10	2.48	0.044		
6/10-12	2.23	0.008		
6/13-14	2.17	0.100		
6/15	Urobilinogen	0.2 mg.		
6/16	Urobilinogen	0.3 mg.		
Stools				
6/14-16	Protoporphyrin, mg./day	1.30		
	Coproporphyrin, mg./day	1.13		

porphobilinogen; quantitative estimations of the porphyrins present were made (Fig. 1 and Table 2).

During the next two months he was treated with meperidine at home by his family physician on several occasions for abdominal colic, and the hydroa returned toward the end of April. He was readmitted to hospital on June 9, 1949, after the onset of jaundice and further severe colic.

Third Admission to Hospital, June, 1949.—His weight on admission was 99 lb. (44.9 kg.); he was markedly icteric with a serum bilirubin of 11 mg. per 100 cc., and on this occasion for a few days his left plantar response appeared to be extensor, the only neurological abnormality, but there was no accompanying weakness. His blood pressure was 130/75. He was noted to have acquired an arthritis of the interphalangeal joints, and movement was painful and impaired (Fig. 3). There were numerous lesions of hydroa aestivale.

No specific therapy was given except for penicillin to control his hydroa, and, by June 21, 1949, his serum bilirubin was down to 1.4 mg. per 100 cc. and he was clinically well apart from minimal lesions of hydroa. He was discharged home on July 11, 1949, having gained 20 lb. (9.1 kg.) in weight while in the hospital.

His urine and serum had fluoresced strongly, as did fluid from the bullous lesions. When he was examined in the dark with ultraviolet irradiation, his entire skin and mucous membranes, and especially scars of old skin lesions, fluoresced a bright red, in striking contrast to the dark blue

fluorescence of a normal person's skin. His urine contained no porphobilinogen, and quantitative estimations of porphyrin content of urine and feces were made (Table 2). His plasma electrolytes appeared to be normal, and renal and hepatic functions were also normal, as judged by the results of urea clearance, galactose-tolerance, and hippuric acid-excretion tests and a cholecystogram. A Kepler test on this occasion, however, showed marked delay in water excretion (Table 1), "ratio A" on two occasions being 13 and 14, respectively.

He was seen as an outpatient on Sept. 7, 1949, and had gained a further 4 lb. (1.8 kg.) in weight. He was normotensive; his serum bilirubin was also normal, and he had no lesions of hydroa. Quantitative porphyrin determinations in urine and feces were again made (Fig. 1).

Fourth Admission to Hospital, November, 1949.—He was next readmitted to hospital on Nov. 14, 1949, with mild abdominal colic and slight jaundice, and he was seen to have lost more

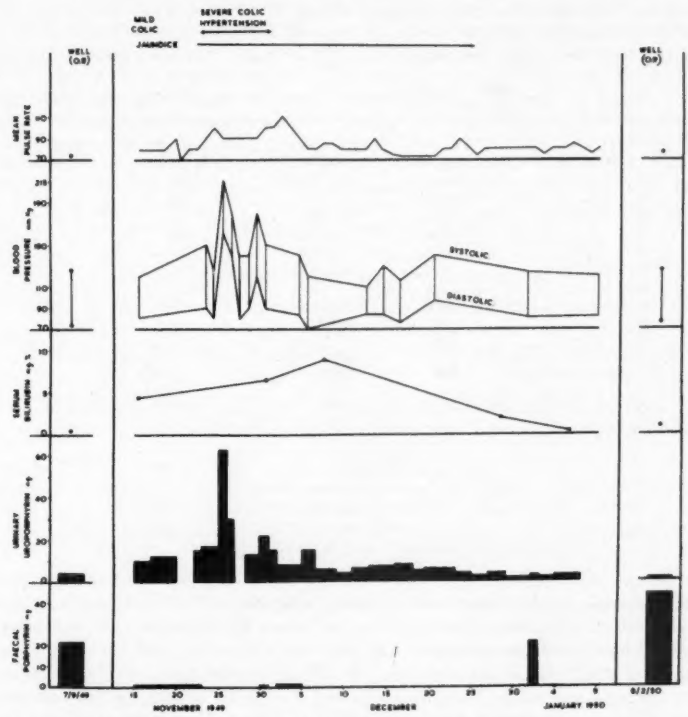

Fig. 4.—Chart illustrating clinical and biochemical features before, during, and after a severe relapse of porphyria cutanea tarda.

than 7 lb. in weight. His jaundice gradually deepened while he was in hospital, his serum bilirubin rising from 4.4 mg. per 100 cc. on admission to 9 mg. on Dec. 7, 1949, and thereafter it remitted, until on Jan. 5, 1950, it was 0.6 mg. per 100 cc. His blood pressure was 120/80 on admission and remained normal for several days. He then, while under observation, had a period lasting from Nov. 23 to 30, 1949, characterized by severe colic, mental confusion, and paroxysmal hypertension, his blood pressure rising initially to 210/160 and remaining elevated for several days. After this episode, he had no further pain or mental confusion, his blood pressure remained normal, and when his icterus had disappeared he was discharged as clinically well on Jan. 9, 1950. Determinations of urinary uroporphyrin and coproporphyrin during this period in hospital revealed a striking parallelism which was followed closely also by the blood pressure (Fig. 4).

Other observations made during this period in hospital showed that the arthritis of his fingers had progressed further (Fig. 3) and was now typically rheumatoid in character. He was also seen to have small juxta-articular crystalline deposits in the skin on the dorsa of his fingers, and biopsy of these showed them to be cholesterol crystals. His serum cholesterol at this stage was 320 mg., and serum uric acid was 3.5 mg. per 100 cc. A 24-hour specimen of urine contained 4 mg. of 17-ketosteroids. A roentgenogram of his hands showed no roentgenologic abnormality. Results of tests of renal and hepatic functions were once again normal, apart from elevation of his serum alkaline phosphatase and bilirubin during the phase of jaundice. Two Kepler tests gave abnormal results (Table 1), but insulin- and dextrose-tolerance curves were normal.

His urine was collected during the whole period of this admission, and at no stage was any porphobilinogen detected. Quantitative daily estimations of porphyrin present were made throughout (Table 3), and several specimens of feces were also sampled and analyzed. The clinical and chemical data relating to this attack are again illustrated in Figures 1 and 4.

The patient remained perfectly well in all respects, and when seen on Feb. 8, 1950, looked as well as he had ever looked and weighed 128 lb. (58.1 kg.). His blood pressure was 125/75;

TABLE 3.—*Analysis of Urines Passed During an Attack, November, 1949*

Period, Hr.	Vol., Ml.	Uroporphyrin, Mg./24 Hr.	Coproporphyrin, Mg./24 Hr.	Total Urobilinogen, Mg.
48	2,200	10.09	0.66	.....
72	2,040	11.91	1.87	.....
24	780	14.18	1.93	< 0.2
48	1,600	16.47	2.66	.....
24	2,050	63.98	11.86	.....
24	1,640	29.55	6.41	.....
48	1,200	13.95	1.01	.....
24	1,800	21.89	1.78	< 0.2
24	1,300	14.92	0.56	.....
72	2,200	9.12	0.45	< 0.2

TABLE 4.—*Analysis of Urine, Feb. 6 and 7 1950, Passed During Remission*

Vol., 1,270 ml.
Total porphyrin, 1.52 mg.
Coproporphyrin, 0.565 mg.
∴ Uroporphyrin, 0.955 mg.
Urobilinogen, < 0.5 mg.

serum bilirubin was 1 mg. and serum cholesterol 180 mg. per 100 cc. His arthritis appeared to be about the same, but his fingers were becoming very stiff, and, despite occupational therapy, he was considerably handicapped. His urine and serum fluoresced strongly, and quantitative urinary and fecal estimations of porphyrin content were made (Fig. 1 and Table 4).

Fifth and Final Admission to Hospital, March, 1950.—On March 10, 1950, however, his parents noticed a remarkable change in his mental state, as he became confused, detached, and at times delirious, with various bizarre hallucinations. He had some abdominal colic, and his urine, instead of being sherry-colored, was deep purple. He was admitted to hospital on March 13, 1950, when it was immediately apparent that his condition was very different from that on any previous occasion; he weighed only 110 lb. (49.9 kg.), a loss of 18 lb. (8.2 kg.) in five weeks. He was very confused and became rapidly more so. He was, at times, frankly manic and required heavy paraldehyde sedation. He had, initially, some colic, but he was not icteric. He had, apart from his mental changes, no demonstrable neurological abnormality, and there was no weakness of any muscle group, alteration in reflexes, or sensory disturbance. He had constant tachycardia (110 to 130 per minute) but was afebrile, and his blood pressure, which was 190/130 on admission, remained elevated until his death. On the evening before his death he was grossly confused, delirious, and manic, and he required paraldehyde sedation and was kept in a restraining sheet. He died suddenly during the night, after the abrupt onset of respiratory paralysis.

His urine on this occasion was port-wine color and, for the first time, gave a positive reaction [6] to Ehrlich's aldehyde test for porphobilinogen. The urine contained no urobilinogen; he was not jaundiced, and his serum bilirubin was 0.5 mg. per 100 cc. His serum alkaline phosphatase was 24 King-Armstrong units and his serum cholesterol 290 mg. per 100 cc. His blood urea on admission was slightly elevated, being 55 mg. per 100 cc. His urine contained a faint trace of albumin.

Necropsy Observations.—A necropsy was carried out (Dr. L. C. D. Hermitte), and the most striking abnormality, apart from the absence of any subcutaneous fat, was the bright pink color of all connective tissues (subcutaneous connective tissue, meninges, pleura, pericardium, costal cartilages, synovial and cartilaginous sufaces of joints, periosteum, aortic intima, endocardium, and valves). Bones deprived of periosteum appeared normal in color.

Most organs were examined under ultraviolet light. The urine fluoresced very strongly, but the cerebrospinal fluid, which appeared normal, showed no fluorescence at all. All fibrous tissues and fibrocartilage fluoresced brightly. The liver showed a generalized patchy pink fluorescence, but the spleen showed none except in the capsule. The cortex and capsule of the kidney fluoresced, as did the pancreas. The lungs fluoresced slightly, but this appeared to be due chiefly to the vessels and bronchi.

The lungs were rather edematous and were engorged with multiple small hemorrhagic areas; otherwise the macroscopic appearance of all organs was normal, with the exception of some congestion of the liver and kidneys. No structural abnormality could be detected on histological examination of any organ, and, in particular, the microscopic appearances of the liver, kidneys, spleen, adrenal glands, testes, heart, lungs, pituitary, brain, spinal cord, intestine, and bone marrow were all normal apart from the pulmonary congestion.

ANTEMORTEM CHEMICAL INVESTIGATIONS

Although a number of cases of acute porphyria have been subjected to more or less detailed chemical examination, only a few cases of porphyria cutanea tarda have been reported (see Gray, Rimington, and Thomson [4] for a review of the literature) and these with but limited chemical data. Gray, Rimington, and Thomson found in their case a remarkable change-over during each attack from a fecal to a urinary route of elimination of porphyrin.

The present patient was kept under observation for 18 months, and during this time he experienced three major attacks (Fig. 1) which were studied in detail from the chemical point of view. Chromatographic methods [5a-c] were employed for the isolation and characterization of porphyrins from the urine and stools and spectrophotometric methods [5d, e] for the following of their quantitative elimination. As a result, a very complete picture of the patient's porphyrin metabolism was obtained and four hitherto undescribed porphyrins were isolated and characterized as their crystalline methyl esters, thus providing the most detailed study yet available of the chemistry of this condition.

Urinary Porphyrin.—The best example of a remission period was the 24-hour specimen collected Feb. 6 and 7, 1950. This gave the analysis shown in Table 4. Paper chromatography showed the presence of porphyrins with eight, five, four, and three carboxyl groups, respectively. All but the uroporphyrin (eight carboxyl groups), being soluble in ether, would be included in the figure for "coproporphyrin."

As an example of excretion during an attack, the analyses shown in Table 3 record the results on the series of urines collected in November, 1949. A paper chromatogram showed the presence of porphyrins with eight, four, three, and two carboxyl groups, respectively. Later during this same attack pentacarboxylic porphyrin was also detected, and at an earlier stage in the illness a heptacarboxylic porphyrin was also found to be present in the urine.

The close parallel existing between the levels of urinary uroporphyrin and coproporphyrin (total ether-soluble porphyrin) during this phase has already been mentioned (see previous section).

Extraction of Total Urinary Porphyrins.—The extraction of the total crude porphyrin from the urine presented considerable difficulty, and various methods were tried on different batches. Adjustment by means of acid to pH 3.0 to 3.2 and storage at 5 C. brought about precipitation of a considerable amount of the porphyrin but was not quantitative. Repeated shaking at this pH

with amyl alcohol was effective but unsuitable when large volumes were being handled. Infusorial earth (*Kieselguhr*) was initially used as an adsorbent at ca. pH 3.5 but replaced later by talc, which was more efficient. It was also found more convenient, when the ether-soluble porphyrin fraction was that especially required, to extract the urines with ether and acetic acid and to discard the extracted residue.

As an example of the technique finally adopted, the extraction will be described of the total porphyrin from 6 liters of urine passed during the attack of March 10 to 20, 1950. The pH was adjusted to 3.5 with acetic acid and the mixture shaken with talc in 30-gm. lots until no more porphyrin was adsorbed. The adsorbent was collected on a Buchner funnel and washed well with water containing a little acetate buffer pH 3.2, and the porphyrin was then eluted by

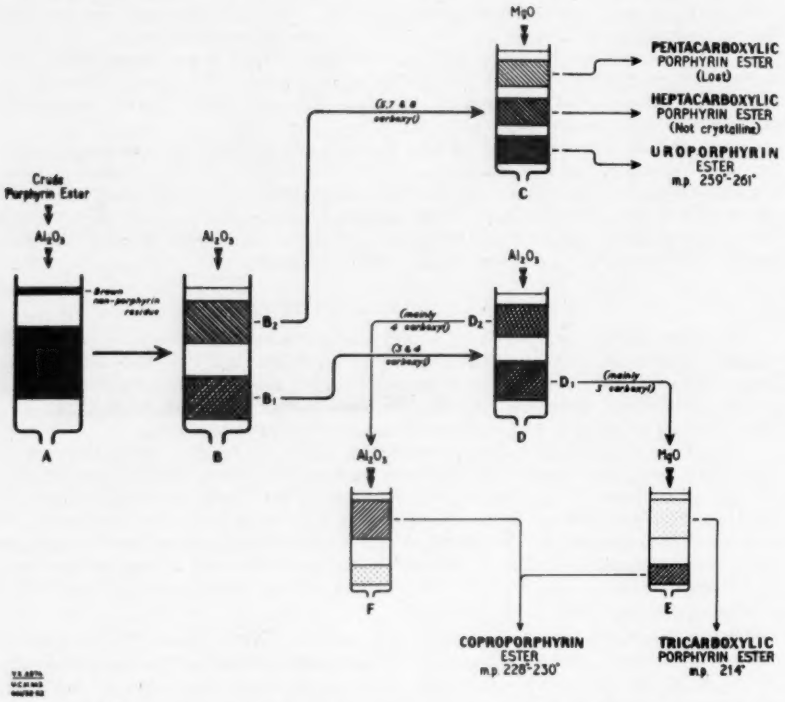

Fig. 5.—Chart illustrating the chromatographic separation of the total urinary porphyrins.

stirring with successive lots of 1% ammonia. The red-brown solution was adjusted to pH 3.1 with hydrochloric acid, and the precipitated porphyrin was collected after chilling overnight. Resolution in 1% ammonia and reprecipitation were repeated five times when the porphyrin solution was deep red and the supernatant fluid practically colorless.

The precipitated porphyrin was dried *in vacuo* and esterified by leaving overnight in methanol previously saturated with hydrochloric acid. To the ester solution was added 2 volumes of chloroform and then, rapidly, with good mixing, sufficient saturated aqueous sodium acetate solution to make the mixture neutral to Congo red. Several volumes of cold water were then added, and, after thorough shaking, the chloroform layer was removed, washed, and dried.

Despite the repeated preliminary reprecipitations, the crude porphyrin still contained some dark brown material. This could be removed either by filtering a solution in anesthetic chloro-

form (containing about 1% ethanol) through a column of magnesium oxide,[7] Grade III,[5a] or by leaving the impure ester, left by evaporation of the chloroform solution, in contact for 24 hours with 25% hydrochloric acid. Under these conditions, the ester is hydrolyzed and the free porphyrin dissolves in the acid, leaving a dark tarry residue which can be washed by stirring with fresh acid and then discarded. The purified porphyrin is precipitated at pH 3.1 and reesterified as described above.

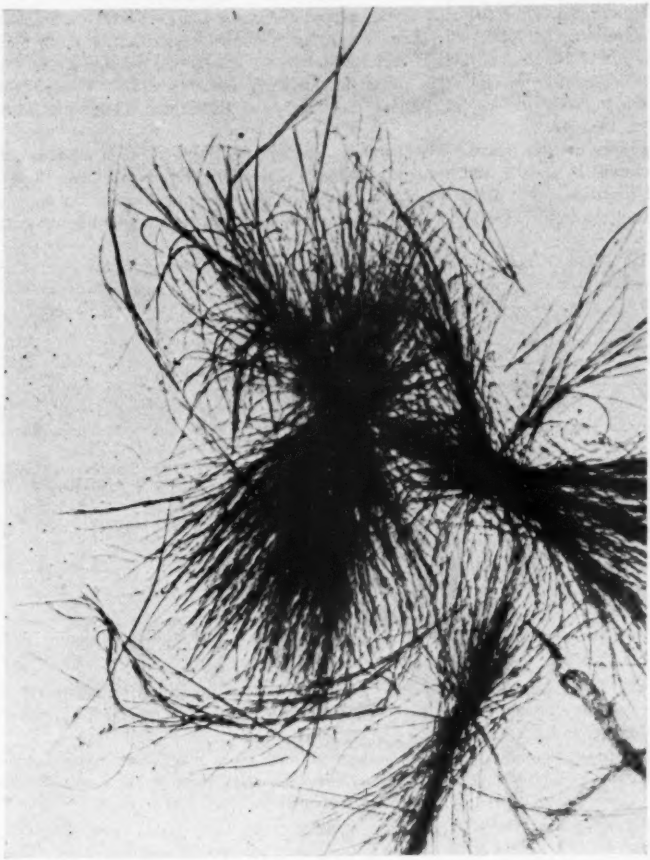

Fig. 6.—Methyl ester of uroporphyrin isolated from urine passed March 19 to 24, 1949, during an attack. ("Waldenström ester" type, m. p., 259-261 C.); × 250.

Chromatographic Separation of Total Porphyrin from Urine.—The total mixed esters were chromatographed upon columns of either aluminum oxide, Grade V, using benzene-chloroform, or magnesium oxide, Grade III, using chloroform-methanol according to the method described by Nicholas,[5a] with the results recorded below and in the flow sheet (Fig. 5). Paper chromatography [5b, c] was employed for the analysis of the fractions.

7. Magnesium oxide and aluminum oxide of graded adsorptive power were standardized according to the technique of Brockmann and Schodder (Ber. deutsch. chem. Gesellsch. **74**:73, 1941).

The preliminary column *A* served to remove a small amount of nonporphyrin pigment, and the second column *B* brought about partial resolution. The lower band (B_1) contained porphyrins with four and three carboxyl functions, and the upper band (B_2), those with eight, seven, and five carboxyl functions. The latter group was successfully separated upon a column of magnesium oxide, but unfortunately the pentacarboxylic porphyrin fraction was lost. The uroporphyrin ester yielded typical hair-like crystals (melting point [m. p.], 259 to 261 C.) from hot chloroform-methanol (Fig. 6). Its microanalysis is recorded in Table 5.

Decarboxylation of this material by the method of Fischer and Zerweck [8] yielded a coproporphyrin mixture (m.p., 202-220 C.) from which, by fractional crystallization, both the I series ester (m. p., 242-245 C.) and the III series ester (m. p., 174-180 C.) were obtained, the former in the greater relative amount. The parent uroporphyrin was thus of the "Waldenström ester" type containing both isomers (cf. Watson, Schwartz, and Hawkinson, 1945,[9] and Nicholas and Rimington, 1951 [5c]).

The porphyrin ester mixture from band B_1 was partially resolved on an alumina column, *D*, into two bands, D_1 and D_2, which were respectively rich in porphyrins containing three and four carboxyl functions. The latter porphyrin was purified further on an alumina column *F* and was crystallized from hot chloroform-methanol, yielding rosettes of needle-like crystals (m. p., 228-230 C.). Paper chromatography showed this material to consist of tetracarboxyl porphyrin alone, and it may be concluded, therefore, that the coproporphyrin isomers I and III were present together in the urine.

TABLE 5.—*Microanalysis of Urinary Uroporphyrin Ester*

	C	H	N	CH_3O
Found	61.16	5.92	6.9	26.85
Uroporphyrin octamethyl ester ($C_{48}H_{54}O_{16}N_4$) requires.....	61.12	5.77	5.9	26.3

TABLE 6.—*Quantitative Relationship Between Fecal Porphyrins*

Date	Clinical State	Total Porphyrin, Mg./Day	"Proto-porphyrin," Mg./Day	"Copro-porphyrin," Mg./Day
June, 1949	Mild attack	2.48	1.30	1.13
Sept., 1949	Well	21.93	6.73	15.20
Nov. 15-22, 1949.....	Attack	0.45	0.23	0.22
Nov. 30, 1949.....	Secondary peak of attack (primary peak was Nov. 25)	0.39	0.12	0.27
Dec. 2-4, 1949.....	Attack subsiding	1.65	0.58	1.07
Jan. 1, 1950.....	Well	21.02	13.10	7.92
Feb. 4-7, 1950.....	Well	44.96	26.45	18.51

The tricarboxylic porphyrin fraction was purified on a magnesium oxide column which removed a small quantity of coproporphyrin. It had a melting point of 214 C., but paper chromatography revealed that traces of coproporphyrin were still present.

Fecal Porphyrins.—The total porphyrin content of the stool varied from about 0.4 mg. per day during periods of attack to as much as 45 mg. per day during remission. Quantitative determinations were made by extracting exhaustively a weighed portion of the well-mixed stool with acetic acid and ether. The ether-soluble porphyrin was then divided into (1) the fraction extracted by 0.1 N hydrochloric acid (called "coproporphyrin" in the table) and (2) that removed only by 1.5 N hydrochloric acid (called "protoporphyrin" in the table). As only a trace of uroporphyrin could be found by chromatographic methods applied to the total porphyrin extract, these figures represent substantially all the porphyrin in the stool. The proportion of "coproporphyrin" to "protoporphyrin" varied markedly in different specimens; in general, there was some tendency for the ratio to be higher during attack than during remission periods (Table 6 and Fig. 1).

8. Fischer, H., and Zerweck, W.: Ztschr. physiol. Chem. **137**:242, 1924.
9. Watson, C. J.; Schwartz, S., and Hawkinson, V.: J. biol. Chem. **157**:345, 1945.

Extraction of Total Fecal Porphyrins.—The most convenient method was found to be as follows: The stool was stirred with several volumes of acetone, and, after some days, the solvent was removed by filtration through a large No. 2 sintered glass funnel. The filtrate contained a considerable quantity of porphyrin which was extracted by adding acetic acid and ether and then diluting with much water. The dehydrated and partially defatted stool residue was ground with acetic acid and shaken with ether, this procedure being repeated until the ether extract was free from red fluorescence. The combined ether extracts, including that from the acetone fraction, were washed repeatedly with 7% sodium chloride solution and finally with water, and the porphyrin was then extracted by shaking with 10% hydrochloric acid. Considerable emulsification occurred, but, by passing a stream of air or nitrogen through the acid, the ether could be removed, leaving a clear acid solution from which precipitated dark fatty

Fig. 7.—Methyl ester of an undescribed monocarboxylic porphyrin (m. p., 177 C.) isolated from stool passed Sept. 4 to 6, 1949, during remission; × 480.

material could be eliminated by filtration. Paper chromatography showed this acid solution to contain porphyrins with four, three, two, and one carboxyl functions, respectively.

Other samples of stool were subjected to an alternative procedure in which, after dehydration with acetone, the fecal residue was extracted by absolute methanol containing 1% sulfuric acid. This removed porphyrins as their methyl esters, which could be transferred to chloroform and further purified. Any ether-insoluble porphyrin would also be extracted by this method, and the final chromatogram did show the presence of the following: eight (trace), five (trace), four, three, and two carboxyl porphyrins, respectively.

Chromatographic Separation of Total Porphyrins from Feces.—Two main studies were carried out, using for the first 33.1 mg. of esters of total ether-soluble porphyrins. In the second, an ether extract of a different fecal specimen was fractionated into a 0.1 N hydrochloric acid fraction (29.5 mg.) and a 1.5 N hydrochloric acid fraction (37.9 mg.), and the porphyrin mixtures from each of these solvents were examined separately.

First Study: Chromatography of the total porphyrin ester upon aluminum oxide removed some brown pigment which was retained at the top of the column and, as the proportion of chloroform to benzene was progressively increased, afforded four bands, bands I to IV, according to the order in which they left the column. Each was rechromatographed to insure homogeneity.

Band I: Paper chromatography showed this to be a monocarboxylic porphyrin. The ester crystallized from chloroform-methanol in long flat plates or prisms with characteristically

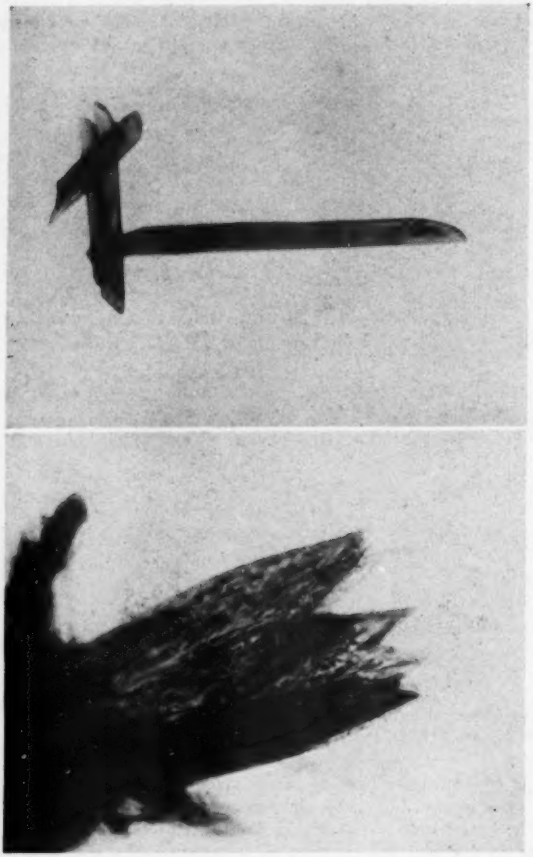

Fig. 8.—Upper, methyl ester of an undescribed dicarboxylic porphyrin (m. p., 165 C.) isolated from stool passed Feb. 4 to 7, 1950, during remission; × 600. Lower, methyl ester of an undescribed tricarboxylic porphyrin (m. p., 193-194 C.) isolated from stool passed Feb. 4 to 7, 1950, during remission; × 650.

rounded ends like dragonflies' wings (Fig. 7). They had a melting point of 177 C. (constant for three recrystallizations) and an etioporphyrin-type absorption spectrum with bands in chloroform at 619.9, 565.3, 531.0, and 498.7 mμ and relative intensities in dioxan of 1.0, 1.43, 2.02, and 2.98. This material does not correspond to any known porphyrin.

Band II: This band consisted of an extremely small quantity of material shown by paper chromatography to be dicarboxylic. The spectrum was etioporphyrin type with bands at 621.6, 566.4, 532.5, and 500.0 mμ.

Band III: This band was preceded by a green zone, and the material it contained was found to be unstable, giving rise readily to a pigment with strong absorption at 640 mμ. Crystallization of the ester afforded a product with the melting point of 179 C., but the absorption spectrum showed bands in chloroform at 640 (strong), 621.0, 576.0, 551.5, and 508.1 mμ. On paper chromatography, a green dicarboxylic spot was present, together with the main tricarboxylic spot. The material appeared to be a tricarboxylic porphyrin which was decomposing into a dicarboxylic porphyrin.

Band IV: This band yielded coproporphyrin III ester (m.p., 146 C.; remelt, 174 C.). Absorption spectrum in chloroform was 621.5, 566.5, 532.1, and 499.5 mμ. Paper chromatography showed only tetracarboxylic porphyrin to be present.

Second Study (0.1 N Hydrochloric Acid Fraction): Chromatography upon aluminum oxide afforded four bands. These were rechromatographed.

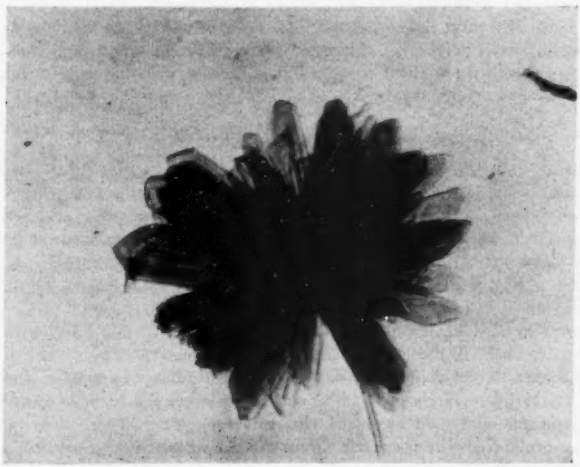

Fig. 9.—Protoporphyrin methyl ester (m. p., 224-227 C.) isolated from stool passed Feb. 4 to 7, 1950, during remission; × 450.

Band I: The porphyrin ester crystallized in slender laths (m.p., 165 C., constant for three recrystallizations, see Fig. 8, upper). Paper chromatography showed it to be a dicarboxylic porphyrin. The absorption spectrum was of etioporphyrin type and had bands in chloroform at 620.5, 566.4, 531.0, and 497.0 mμ, and relative intensities in dioxan of 1.0, 1.36, 1.92, and 3.0. This material does not correspond to any known porphyrin.

Band II: This band consisted of only a trace of dicarboxylic porphyrin with a band in chloroform at 622.0 mμ.

Band III: The porphyrin ester crystallized in lanceolate plates or prisms with a melting point of 193 to 194 C. (Fig. 8, lower). Paper chromatography showed the presence of only tricarboxylic porphyrin, but the substance appeared to be unstable, since a residue left after a melting-point determination and subsequently hydrolyzed for paper chromatography contained some dicarboxylic porphyrin in addition to the original tricarboxylic porphyrin. The absorption spectrum was of etioporphyrin type with bands in chloroform at 621.0, 566.4, 532.5, and 499.2 mμ, and relative intensities in dioxan of 1.0, 1.43, 2.20, and 3.1. This material does not correspond to any known porphyrin.

Band IV: This band yielded coproporphyrin III ester.

Second Study (1.5 N Hydrochloric Acid Fraction): The crude ester was resolved upon aluminum oxide into three bands. These were rechromatographed.

Band I: This band was evidently a mixture and was, therefore, run on a magnesium oxide (Grade III) column. On this adsorbent it yielded an upper band, the porphyrin of which crystallized in fine rosettes (m.p., 224-227 C.; Fig. 9). It was a pure dicarboxylic porphyrin with bands in chloroform at 630.5, 575.0, 540.6 and 505.0 mμ and identical, therefore, with protoporphyrin IX dimethyl ester. The lower band on the magnesium oxide column contained a porphyrin crystallizing in elongated flat plates or prisms (m.p., 194-195 C.; constant for three recrystallizations). Paper chromatography showed it to be a dicarboxylic porphyrin. Its absorption spectrum was of etioporphyrin type and had bands at 620.7, 566.0, 532.0 and 499.0 mμ, and relative intensities in dioxan of 1.0, 1.45, 2.08, and 3.14. The above data for spectrum and melting point do not correspond with any of the known dicarboxylic porphyrin esters.

Bands II and III: These bands contained too small an amount of porphyrin for crystallization. By paper chromatography and spectral absorption measurements they were shown to correspond, respectively, with the unstable tricarboxylic porphyrin and coproporphyrin.

Serum Porphyrins.—Samples of serum obtained during attack periods exhibited a bright red fluorescence under ultraviolet light. Several methods were tried, unsuccessfully, in an endeavor to isolate the responsible porphyrins. Since the acetic acid-ether technique described by Fisher failed to extract more than minimal quantities, it is probable that the porphyrins present were of the ether-insoluble uroporphyrin type.

POSTMORTEM CHEMICAL INVESTIGATIONS

Bone Marrow.—Marrow scrapings were defatted and then soaked in 1.5 N hydrochloric acid. The extract was shaken with ether to remove further fat, then made neutral to Congo red by addition of sodium acetate. No porphyrin was extracted by shaking with ether; however, it was removable by ethyl acetate. From this solvent it was returned to dilute hydrochloric acid and chromatographed upon paper. Only uroporphyrin was present.

Liver.—The minced tissue (1,300 gm.) was extracted by repeated mechanical shaking with acetic acid and ether, followed by filtration on a large Buchner funnel. Ether was removed from the extract by distillation and the residue (380 ml.) poured into 1.5 N hydrochloric acid (2 liters). After shaking, the brown flocculent precipitate was removed, the filtrate shaken with light petroleum to remove fat, and the porphyrin then transferred to ether, and thence, after washing, to 1.5 N hydrochloric acid. The purplish solution was shaken with chloroform to remove bile pigment and then examined chromatographically. There were present coproporphyrin and pentacarboxylic porphyrin. From the chloroform some extracted porphyrin was recovered, and this proved to be protoporphyrin.

The tissue residue, after acetic acid-ether extraction, was suspended in dilute ammonia solution and the dark brown extract adjusted to pH 3.1 and shaken with ethyl acetate. Some ether-insoluble porphyrin was removed by this solvent and shown by paper chromatography to consist of uroporphyrin, together with heptacarboxylic porphyrin.

A small portion of liver was homogenized and tested separately for porphobilinogen but with negative result.

Kidney.—This organ (115 gm.) was subjected to the same treatment as that described for the liver. Uroporphyrin, coproporphyrin and a pentacarboxylic porphyrin were present.

Spleen.—By the same method 155 gm. yielded evidence of coproporphyrin and also some ether-insoluble porphyrin.

Bile.—About 12 ml. of dark viscid bile was available. It was extracted thoroughly with acetic acid and ether and the residue then shaken with 3 N hydrochloric acid. In the latter extract uroporphyrin was shown to be present and in the former coproporphyrin and pentacarboxylic porphyrin.

Urine.—The bladder contained 180 ml. of urine. This was analyzed and found to contain porphobilinogen (2 +); 48.8 mg. per liter of uroporphyrin before heating; 78.2 mg. per liter of uroporphyrin after heating, and 5.1 mg. per liter of coproporphyrin.

COMMENT

The clinical and chemical findings reported in this case justify its being considered an example of porphyria cutanea tarda. Thus, there was light photosensitivity associated with periodic attacks of colic succeeded by jaundice. Uroporphyrin (of Waldenström ester type; ester m. p., 259-261 C.) was present in the urine, together with increased coproporphyrin (Series I and III); a pentacarboxylic and a tricarboxylic porphyrin were also detected. Porphobilinogen was absent from the urine until the terminal attack, which differed in several respects from previous ones. Nervous symptoms were absent, although the subject was mentally rather backward; however, psychotic disturbances appeared during the terminal attack coincident with the positive porphobilinogen test. Porphyrin was noted in the serum during attacks, and there was a tendency to fragility of the skin.

The reciprocal relationship between the urinary and the fecal porphyrin and the deviation of the route of excretion from the stool to the urine during attacks, a phenomenon first noticed by Gray, Rimington, and Thomson [4] in their case, were established in the present case also by observation over an extended period. Precisely similar behavior has been observed by one of us (C. R.) in two other cases of porphyria cutanea tarda since this work was completed, and the conviction is growing that this urinary-fecal reciprocal relationship, associated with phases of the disease, must be regarded as a highly characteristic feature of porphyria cutanea tarda. Reference to the condition as a "mixed type" of porphyria is, therefore, undesirable.[10]

The deviation of pigment excretion from the intestine to the kidney would suggest a temporary hepatic insufficiency. The serum bilirubin showed a gradual rise during each attack but appeared to lag somewhat behind the rise and fall of the urinary porphyrin. The nature of this hepatic dysfunction is obscure. Galactose-tolerance and hippuric acid-excretion tests showed no abnormality, even when the serum bilirubin was 7 mg. per 100 cc.; nevertheless, it must be remembered that these tests measure functions of the liver other than that of biliary excretion. In our opinion, the findings could most suitably be explained by postulating spasm of the common bile duct and sphincter brought about by some toxic substance. This might be porphyrin itself or possibly some pyrrolic precursor, or it might be an endogenous material unrelated to pigment metabolism. An example of a pure chemical material inhibiting the elimination of bile by the liver without causing other recognizable damage is to be seen in the plant material "icterogenin" described by Rimington and Quin in 1937.[11] That liberation of some substance causing vascular spasm occurred during attacks is suggested not only by the hypertension from which the patient suffered but also by the observed narrowing of the retinal arteries. Much work has been done in the past upon the pharmacology of porphyrins,[12] and spasm of plain muscular structures has been attributed to them.

Since large quantities of porphyrin are being regularly eliminated by these patients in the bile, episodes of jaundice, with consequent accumulation of porphyrin in the circulation, will readily account for the photosensitivity suffered periodically

10. Watson, C. J.: Lancet **1**:539, 1951.

11. Rimington, C.; Quin, J. J., and Roets, G. C. S.: Onderstepoort J. Vet. Sc. **9**:225, 1937.

12. Vannotti, A.: Porphyrine und Porphyrinkrankheiten, Berlin, Julius Springer, 1937.

by patients with porphyria cutanea tarda. We are as yet entirely ignorant of the cause of these exacerbations.

Some observations were made on our patient in June, 1949, in an attempt to clarify the mechanism of the photosensitivity. At the time, he had minimal lesions of hydroa aestivale, the acute episode having subsided. Three test areas were exposed for 20 minutes to strong sunlight, the first being left uncovered, the second being protected by a Wood filter, and the third by a sheet of ordinary window glass. A marked erythema occurred in the unprotected area and in that covered by plain glass, but no reaction whatsoever resulted in the second area. Subsequently no lesions of hydroa developed in these areas. A week later, similar exposure to strong sunlight was carried out for one hour, a graded series of Wratten filters being used. By far the greatest erythema developed below the Wratten G (15) filter, which transmits appreciably only wavelengths greater than 540 mμ, the reaction approximating that in the control uncovered area. Less erythema developed below the filters that transmitted chiefly above and below this wavelength. No other reaction occurred than erythema, and no erythema developed after exposure at 18 in. (45.7 cm.) to a 100-watt electric bulb for one hour. These findings would seem to indicate that the wavelength of light that precipitates the lesions of hydroa is most probably in the visible spectrum and is probably greater than 500 mμ. Taylor, Solomon, Weiland, and Figge in 1946 [13] were not able to induce lesions in their patient with chronic porphyria by exposure to ultraviolet irradiation. Our patient was repeatedly examined under ultraviolet light, but no new lesions developed which could be ascribed to this procedure. Gray, Rimington, and Thomson [4] were able to induce bullous lesions by tapping the skin repeatedly with a stick, showing that trauma might be a causal factor for the skin lesions. Repeated light tappings in a single area with a ruler, prolonged for over 10 minutes, were not followed in our patient by any abnormality, even when the procedure was carried out at a time that fresh lesions of hydroa were developing.

The association between alterations in body weight and relapses and remissions in acute porphyria was pointed out by Discombe and D'Silva,[14] who noted that a very marked fall in body weight preceded each relapse. It is difficult to analyze the cause of this phenomenon, but we suggest that it is probably closely related to a disturbance of renal or adrenal cortical function, which, as we shall show, appears to be an integral part of this disease. A similar phenomenon is apparent in our patient, in whom marked loss of weight was noted during both of his last two relapses, in November, 1949, and March, 1950, as can be seen from Figure 1.

The role of the kidney and of the adrenal cortex in fashioning the pattern of this disease is obscure. Changes in electrolyte metabolism in acute porphyria were thought by Linder [15] and Abrahams, Gavey, and Maclagan [16] to suggest adrenal cortical failure, but Prunty [17] showed that this hypothesis was unlikely to be tenable and concluded that many of the changes could be ascribed to faulty renal function,

13. Taylor, I. J.; Solomon, M. L.; Weiland, G. S., and Figge, F. H. J.: J. A. M. A. **131**:26, 1946.

14. Discombe, G., and D'Silva, J. L.: Brit. M. J. **2**:491, 1945.

15. Linder, G. C.: Lancet **2**:649, 1947.

16. Abrahams, A.; Gavey, C. J., and Maclagan, N. F.: Brit. M. J. **2**:327, 1947.

17. Prunty, F. T. G.: J. Clin. Invest. **28**:690, 1949.

a suggestion also made by Davies.[18] It is perhaps significant that patients with demonstrably normal renal function have not shown abnormalities of electrolyte metabolism.[19] In our patient there was at no stage any abnormality in the serum electrolyte level, but balance studies were not carried out. All renal-function tests always gave normal results.

We are, however, on less sure ground when we consider the role of the adrenal cortex. In our patient the only completely normal results on Kepler tests of water excretion (Table 1) were those of Oct. 25, 1948, and Nov. 15, 1949, the latter being performed just before a severe relapse. A further observation three weeks later again showed delay in water excretion, and it seems, therefore, likely that there was in this patient a phasic variation of adrenal cortical efficiency. Unfortunately, more specific tests of adrenal cortical function, such as those described by Forsham, Thorn, Prunty, and Hills,[20] were not carried out; we feel, nevertheless, that in this patient there was definite adrenal cortical dysfunction, and that this was, in certain respects, a deficiency syndrome. Prunty [17] showed that during the active phase of acute porphyria adrenal activity was increased and that there was histological evidence that secretory activity may be increased.

The fundamental work of Hench, Kendall, Slocumb, and Polley [21] has shown that the changes of rheumatoid arthritis are potentially reversible and appear to be related to a deficiency which can be remedied by administering a hormone derived from the adrenal cortex. The precise role of the adrenal capsules in the genesis of rheumatoid arthritis is obscure, but the latter is not commonly observed in patients with Addison's disease.

In our patient, however, we were able to observe over a period of months the development of a true arthritis of rheumatoid type (Fig. 3) at a time that the patient was manifesting other evidence of adrenal cortical deficiency, such as abnormal results on Kepler tests and low 17-ketosteroid excretion. We are not in a position to put forward any theory regarding the causation of this arthritis. As is usual, however, in patients with rheumatoid arthritis there was no histological evidence of adrenal abnormality at the autopsy, so the defect was apparently rather in the quantity or nature of the constituents of the adrenal cortical hormone that was being produced.

A further point of considerable interest that emerged from our study of this patient was that there appeared to be at times a direct correlation between the incidence of hypertension and the excretion of urinary uroporphyrin by the patient. This correlation can be seen in Figure 4, where the attack of November, 1949, is shown in detail, and in Figure 10, where typical results at different phases of the disease are charted. It is not easy to determine whether the observation has been noted before, as it is seldom possible to correlate clinical blood-pressure observations with chemical estimations of urinary porphyrin excretion. Prunty [17] has reported

18. Davies, D.: Brit. M. J. **1**:846, 1949.

19. Grossfield, E.: Brit. M. J. **1**:1240, 1951.

20. Forsham, P. H.; Thorn, G. W.; Prunty, F. T. G., and Hills, A. G.: J. Clin. Endocrinol. **8**:15, 1948.

21. Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Proc. Staff Meet., Mayo Clin. **24**:181, 1949.

on electrolyte metabolism in a case of acute porphyria, and the same case is Case 1 in the paper by McSwiney, Nicholas, and Prunty.[22] Reconsideration of the data presented there shows that in this case also a rough relationship is apparent. Hypertension was observed from Day 14 to 24, and thereafter the blood pressure tended to fall. Urinary studies showed high urinary porphyrin levels from Day 15 to 24. The next observations on Days 29, 33 to 35, and 47 showed progressively lower levels recorded. In the case studied by Discombe and D'Silva,[14] high urinary excretion of uroporphyrin was noted during the fourth attack, but reference to the original notes gives no clue to blood-pressure readings during this period,

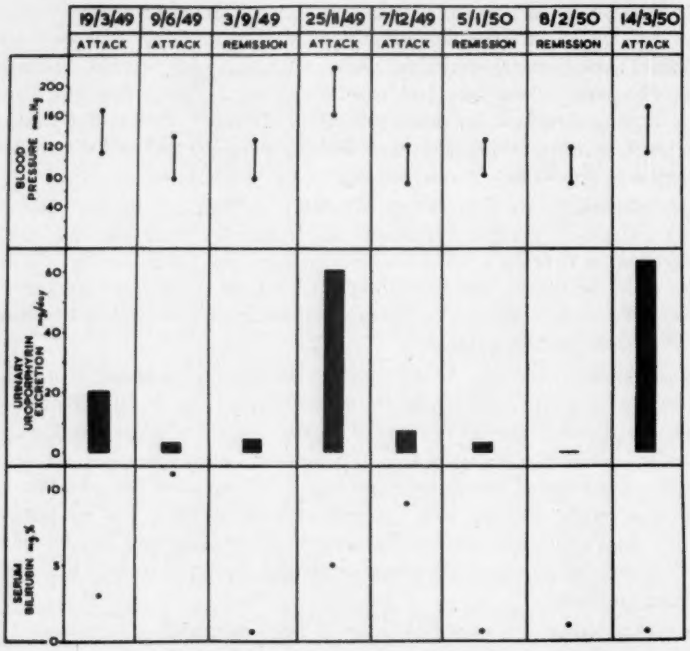

Fig. 10.—Chart illustrating relationship of arterial blood pressure to urinary uroporphyrin excretion and to serum bilirubin on selected days during remission and relapse over a period of a year.

although episodic hypertension was noted on other occasions, the blood pressure during the sixth attack being 160/130, whereas in remission it was recorded as being 124/72. In the case of acute porphyria described by Grossfield,[19] the urine terminally contained 285 mg. of uroporphyrin per liter, and death was preceded by an earlier rise in the patient's blood pressure. Paroxysmal hypertension was not recorded by Gray [23] in a case of acute porphyria in which episodic high excretions of urinary uroporphyrin were noted.

22. McSwiney, R. R.; Nicholas, R. E. H., and Prunty, F. T. G.: Biochem. J. **46**:147, 1950.
23. Gray, C. H.: Arch. Int. Med. **85**:459, 1950.

Rimington [24] noted a correlation of pulse rate and porphyrin excretion in the case studied by Discombe and D'Silva [14]; such a correlation is roughly apparent in Figure 1 with respect to our case, although it is not close.

Taylor, Solomon, Weiland, and Figge [13] noted that the skin lesions in their patient fluoresced strongly under near-ultraviolet light, as also did the tongue and sebaceous secretions. A most striking feature in our patient was the way in which his whole skin, mucous membranes, tongue, and vesicular lesions fluoresced a bright orange-pink. The light used emitted radiation extending from 3130 to 4070 A, with 95% of the radiation concentrated at 3650 A. His teeth and nails did not fluoresce abnormally, but it was noted that his gums, and any deposit upon his teeth, fluoresced very brightly. Scars of old vesicular lesions showed a more intense fluorescence. This demonstration of in vivo fluorescence was extremely dramatic and sufficiently striking to justify the use of ultraviolet light examination in any case in which disturbance of porphyrin metabolism is suspected. The use of this method in autopsy examinations and in biochemical procedures is well established, but it is not commonly used as a diagnostic procedure, despite the advice of Figge.[25]

The chemical findings in the present case are of particular interest in revealing once again the complexity of the porphyrin excretion pattern. Porphyrins with seven and six or five carboxyl groups per molecule have been shown to accompany uroporphyrin and coproporphyrin in urines from persons with congenital porphyria [26] and acute porphyria.[27] The appearance of such hitherto unrecognized porphyrins also in porphyria cutanea tarda implies that there must exist a little-explored intermediary metabolism of the porphyrin pigments. In the present instance, four new porphyrins have been isolated, namely, a monocarboxylic porphyrin (ester m. p., 177 C.), two differing dicarboxylic porphyrins (ester m. p.'s, 165 and 194-195 C., respectively), and an unstable tricarboxylic porphyrin (ester m. p., 193 C.) which readily passes into a dicarboxylic porphyrin. It was impossible to determine the chemical structure of these pigments or the isomeric series to which they belonged, since only minimal amounts were available; nevertheless, it is believed that they were obtained in pure form. The nearness of the melting point (165 C.) of one of the dicarboxylic porphyrin esters to that of mesoporphyrin I (170 C.) prompted us to perform mixed melting-point determinations with synthetic mesoporphyrin I dimethyl ester (kindly made available by Dr. W. Siedel). Depression of the melting point proved nonidentity of the materials.

If, in the course of erythropoiesis, the first porphyrin to be formed is a uroporphyrin, then the porphyrins with fewer carboxyl groups must be formed from it in the course of metabolism. In the pathological subject, metabolic blocks could arise at one or more stages. It is of much interest, in this connection, that the porphyrins eliminated by our patient in the stool during remission were mainly protoporphyrin IX and coproporphyrin III, whereas, during "attacks" or exacerbations of the disease, the chief product was uroporphyrin (isomers I and III) eliminated together with much coproporphyrin in the urine. There would thus

24. Rimington, C.: Biochem. J. **37**:443, 1943.

25. Figge, F. H. J.: Bull. School Med. Univ. Maryland **26**:165, 1942.

26. Rimington, C., and Miles, P. A.: Biochem. J. **50**:202, 1951.

27. Nicholas and Rimington.[5c] McSwiney, R. R.; Nicholas, R. E. H., and Prunty, F. T. G.: Biochem. J. **46**:147, 1950.

appear to have been a persistent overproduction of porphyrin with, at times, an impairment of the processes whereby transition is effected from uroporphyrin to protoporphyrin. It is also possible that the ratio of the isomer types may have been subject to variation—factors all of which combined in forming the clinical picture of this case of porphyria cutanea tarda.

Full clinical and biochemical studies of further cases of this interesting syndrome will probably clarify some of the features which are at present obscure. Another promising line of approach would be a reinvestigation of the pharmacology of porphyrins, using really pure pigments belonging to both the I and the III series of isomers.

SUMMARY

A case of porphyria cutanea tarda was studied for 18 months, during which time the patient experienced three major attacks, the last of which proved fatal.

The clinical features accompanying the attacks included photosensitivity, abdominal colic, paroxysmal hypertension, and recurrent jaundice. There was progressive development of arthritis of the hands, and the patient displayed some evidence of adrenal cortical dysfunction.

Investigation of the pattern of porphyrin excretion showed a reciprocal relationship between the amounts of porphyrin excreted in the urine and the amounts excreted in the feces. During each attack, considerable quantities of uroporphyrin (methyl ester melting point [m. p.], 259 to 261 C., yielding both coproporphyrins I and III on decarboxylation) and of coproporphyrin were excreted in the urine, together with smaller quantities of unidentified porphyrins containing seven, five, and three carboxyl groups, respectively. Porphobilinogen was present during only the last, fatal attack. Fecal porphyrin during each attack dropped to about 0.5 mg. a day. During remission periods the fecal porphyrin, which was mainly protoporphyrin, rose to 20 to 40 mg. a day, while the urinary porphyrin was correspondingly reduced.

As a result of the application of newer techniques for the chromatographic separation of porphyrin mixtures, four hitherto undescribed porphyrins having one, two, and three carboxyl groups, respectively, were isolated and their methyl esters characterized by chromatographic behavior, crystalline form, melting point, and absorption spectrum.

In this case, there appeared to be a relationship between urinary uroporphyrin excretion and the arterial blood pressure.

The characteristics distinguishing porphyria cutanea tarda are discussed, and this condition is compared with other diseases of the porphyria group.

Prof. E. J. Wayne and Dr. I. B. Sneddon gave permission to publish details of this case. Miss Phyllis Miles gave technical assistance with the porphyrin estimations, and Dr. Arthur Jordan arranged for other observations to be carried out by his Department of Biochemistry. Mr. J. Smiles, of the National Institute for Medical Research, Mill Hill, made photographs of the crystalline preparations. The Hartridge reversion spectroscope and Beckman photoelectric spectrophotometer were purchased from a grant to one of us (C. R.) from the London University Research Grant Fund.

OBSERVATIONS ON ALLEGED SEROTONIN- (ENTERAMINE)-LIKE NATURE OF CEREBRAL PRESSOR SUBSTANCE OF TAYLOR, PAGE, AND CORCORAN

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IN AN IMPORTANT article which appeared in this journal, Taylor, Page, and Corcoran [1] described the liberation into the blood stream of a vasopressor substance on many forms of neural stimulation in the dog (centripetal vagus stimulation, centripetal stimulation of the cut sciatic nerve).

This substance is believed to be distinct from epinephrine, arterenol, renin, angiotonin, and vasopressin (pitressin®). Since its activity is inhibited and ultimately abolished by 1-hydrazinophthalazine (C-5968), and since this drug also inhibits the pressor action of serotonin, these authors are of the opinion that serotonin may be "the type substance of a new and important series of pressor compounds, of which the cerebral pressor substance is one."

Taylor and his associates hold also the view that this substance, which provisionally is believed to be of nerve origin, may participate in varying degree in more than one form of hypertensive disease.

Similar and perhaps identical to the vasopressor principle described by the Cleveland researchers is "arteriohypertensin," [2] which is believed to be liberated into the blood on stimulation of the central end of the vagus from a precursor "arterin" contained in the arterial walls.

The studies carried out on enteramine in this Institute over many years may help clear up the problems posed by Taylor and associates—above all, the problem of the similarity between their cerebral vasopressor substance and serotonin.

The most important results obtained in the course of our investigations may be summarized as follows:

1. Enteramine is the specific deposit or secretion product of the enterochromaffin cell system (it occurs in typical enterochromaffin cells of the gastrointestinal mucosa of vertebrates and ascidians, in chromaffin cells of the posterior salivary glands of Octopoda and of the hypobranchial body of Muricidae, in part of the chromaffin granular cells of the cutaneous glands of Amphibia, in enterochromaffin-like cells of the thymus of birds, etc.).[3]

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1. Taylor, R. D.; Page, I. H., and Corcoran, A. C.: A Hormonal Neurogenic Vasopressor Mechanism, A. M. A. Arch. Int. Med. **88**:1, 1951.

2. Diaz, C. J.; de la Barreda, P.; Candeira, J. S., and Alcala, R.: Additional Studies on the Function of the Vascular Wall in Arterial Pressure Regulation, Bull. Inst. M. Res., Univ. Madrid **3**:131, 1950.

3. Erspamer, V., and Asero, B.: L'enteramina, prodotto ormonale specifico del sistema enterocromaffine, Ricerca scient. e ricostruz. **21**:2132, 1951; Identification of Enteramine, the Specific Hormone of the Enterochromaffin Cell System, as 5-Hydroxytryptamine, Nature, London **169**:800, 1952.

2. Among the several pharmacological properties possessed by enteramine [4] (antidiuretic action in hydrated rats; action on the systemic blood pressure, hypertensive or hypotensive according to circumstances; positive inotropic, chronotropic, and tonotropic action on the heart of mollusks; stimulant action on the urinary bladder of the dog, both isolated and *in situ;* stimulant action on the estrous uterus of the rat and on the duodenum of the same animal, etc.), the action which we believe to be, at least in mammals, by far the most important and elective is its antidiuretic action. Enteramine antidiuresis is due to a reduction of the glomerular filtration rate, as a consequence of a fall in the intraglomerular hydrostatic pressure. This fall, in its turn, is caused by an increase in tonus or a spasm of the contractile structures of the afferent vascular bed of the glomerulus.[5] The intrarenal vascular point of attack of enteramine may also be easily demonstrated by visualizing the renal vessels with benzidine or thioflavine-S.

Massive doses of enteramine cause a more or less diffuse ischemic necrosis of the cortex of the kidney.

On the basis of all our experimental data we consider enteramine as a true hormone regulating the intrarenal vascular tonus and, consequently, the blood flow through the kidney.

Doses of enteramine of as little as 5 γ per kilogram of body weight affect diuresis in hydrated rats, and similar doses are probably also active in man. Such doses in the rat are at least 10,000 times less than the median lethal dose and 50 to 100 times less than the dose capable of modifying the systemic blood pressure.

3. Enteramine has recently been isolated as the pure picrate from extracts of posterior salivary glands of Octopus vulgaris and from extracts of the skin of Discoglossus pictus.[6] The two natural products have proved to be identical and also to be identical to 5-hydroxytryptamine, which we obtained by synthesis [7] contemporaneously with Hamlin and Fischer,[8] and with Speeter, Heinzelmann, and Weisblat.[9]

Serotonin, as described by Rapport, Green, and Page,[10] is circulating enteramine. A physiological participation of serotonin in extrarenal vascular regulation

4. Erspamer, V.: Enteramina e 5-metossitriptamina: Tossicità; azione sulla diuresi, sulla pressione del sangue e su alcuni organi a muscolatura liscia, Ricerca scient. e ricostruz. **22**:694, 1952.

5. Erspamer, V., and Ottolenghi, A.: Antidiuretic Action of Enteramine, Experientia **6**:428, 1950; Preliminary Researches on the Mechanism of the Antidiuretic Action of Enteramine, ibid. **7**:191, 1951; Antidiuretic Action of Small Doses of Enteramine Extracts in the Rat, ibid. **8**:31 and 152, 1952; Antidiuretic Action of Pure Synthetic Enteramine in Hydrated Rats, ibid. **8**:232, 1952.

6. Erspamer, V., and Asero, B.: Isolation of Enteramine from Extracts of Posterior Salivary Glands of Octopus Vulgaris and of Discoglossus Pictus' Skin, J. Biol. Chem., to be published.

7. Asero, B.; Colò, V.; Erspamer, V., and Vercellone, A.: Synthese des enteramins (5-oxytryptamin), Ann. chem. **575**:69, 1952.

8. Hamlin, K. E., and Fischer, F. E.: The Synthesis of 5-Hydroxytryptamine, J. Am. Chem. Soc. **73**:5007, 1951.

9. Speeter, M. E.; Heinzelmann, R. H., and Weisblat, D. I.: The Synthesis of the Blood Serum Vasoconstrictor Principle Serotonin Creatinine Sulfate, J. Am. Chem. Soc. **73**:5514, 1951.

10. Rapport, M. M.; Green, A. A., and Page, I. H.: Crystalline Serotonin, Science **108**:329, 1948; Partial Purification of the Vasoconstrictor in Beef Serum, J. Biol. Chem. **174**:735, 1948; Serum Vasoconstrictor (Serotonin): III. Chemical Inactivation, ibid. **176**:1237, 1948; IV. Isolation and Characterization, ibid. **176**:1243, 1948. Rapport, M. M.: Serum Vasoconstrictor (Serotonin): V. The Presence of Creatinine in the Complex; A Proposed Structure of the Vasoconstrictor Principle, ibid. **180**:961, 1949.

has as yet not been demonstrated. The liberation of serotonin at the time of clotting of blood may have no particular physiological significance, but may simply depend on the destruction of the lighter elements of the blood, probably the thrombocytes (platelets), in which the substance seems to be prevailingly contained.[11]

The remarkably high enteramine content of the mammalian spleen [12] may also be due to an extensive destruction of thrombocytes in this organ.

4. None of the numerous indole derivatives tested up to now can compete with enteramine in its antidiuretic action (tryptamine, 1-methyltryptamine, N-methyltryptamine, N, N-dimethyltryptamine, 5-methoxytryptamine, tryptophane, tryptophanol, hypophorine, bufotenin, bufotenedine, bufothionine, dehydrobufotenin, bufoviridine). 5-Methoxytryptamine, the most powerful of the enteramine-like compounds which we have at our disposal, is 2 to 4 times less active than enteramine, and bufotenin (N, N-dimethylenteramine) is about 10 times less active.[12a]

In our recent work we have repeatedly emphasized the necessity of taking seriously into account the distribution of enteramine and of the enterochromaffin cell system in the whole animal kingdom, when attempting to interpret the physiological significance of this substance.

This introduction has been necessary to allow me to discuss the fundamental problem of the present paper: Is there sufficient evidence to include the cerebral vasopressor principle in the group of serotonin-like substances?

The only points of resemblance between the cerebral vasopressor substance and serotonin, as set forth by Taylor and associates, are their common "pure" hypertensive action and their similar behavior toward 1-hydrazinophthalazine.

What value may be ascribed to these resemblances?

EXPERIMENTAL RESULTS

A. *Action of Enteramine (Serotonin) on the Systemic Blood Pressure.*—The Cleveland researchers consider serotonin a "pure" pressor agent. This is inexact, even for the dog. Indeed, if it is true that the substance in this animal causes at the most moderate pressor rises (as a rule unsatisfactorily proportional to the dose and frequently preceded by a transient hypotension), it is equally true that the response may be decidedly hypotensive, or else biphasic, with an initial pressure fall followed by a moderate rise.

This pattern is seen at the beginning of the experiment, that is, in the fresh animal. As the experiment progresses and the injections of enteramine or enteramine-like substances are frequently repeated, the pressor responses to enteramine become more and more unpredictable and irregular. Pure hypertensive responses are rare, while, on the contrary, the biphasic (hypertension-hypotension) or the decidedly hypotensive reactions are common.

11. Rand, M., and Reid, G.: Source of "Serotonin" in Serum, Nature, London **168**:385, 1951.

12. Erspamer, V.: Pharmakologische Studien über Enteramin: Über das Vorhandensein eines enteraminähnlichen Stoffes in Milzextrakten, Arch. exper. Path. u. Pharmakol. **196**:391, 1940.

12a. Erspamer, V.: Biological Activity of Some Enteramine-Related Substances, Nature, London **170**:281, 1952.

The anesthetized cat is not very sensitive to enteramine. Small doses cause a predominantly hypotensive action; with larger doses hypotension may be followed by a moderate pressor rise, which is lower than that provoked by equal doses of tryptamine and by doses of epinephrine 100 times smaller.

In the spinal cat, enteramine always possesses a hypertensive action which is moderate in any case and at least 20 to 30 times lower than that of epinephrine.

The rabbit reacts to enteramine with a constant pressure fall. This hypotensive response, already described in 1940 for crude enteramine extracts,[13] has thus been confirmed also for pure enteramine.

The guinea pig gives modest and irregular responses, in most cases biphasic: the hypotensive peak is followed by a transient pressor rise.

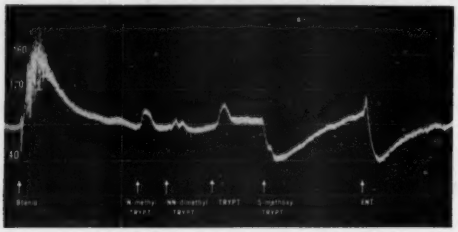

Fig. 1.—Blood pressure of dog weighing 7 kg., anesthetized with pentobarbital. Two hours after start of experiment, in which repeated injections of different indolalkylamines were given, 1,000 γ of enteramine picrate (*ENT*) and of 5-methoxytryptamine hydrochloride provoked a conspicuous fall of blood pressure; 100 γ of bufotenedine hydroiodide (*Btenid*) and 2,000 γ of tryptamine hydrochloride, N-methyltryptamine hydrochloride, and N,N-dimethyltryptamine hydrochloride still elicited, on the contrary, hypertensive responses. Time is in minutes.

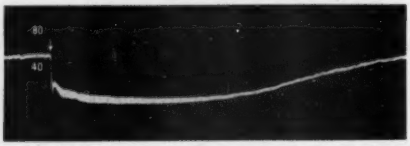

Fig. 2.—Blood pressure of rabbit weighing 2.5 kg., anesthetized with urethan. At the arrow intravenous injection of 2,000 γ enteramine picrate was started. Substance elicited an intense, pure hypotensive reaction.

In the intact nonanesthetized rat, the subcutaneous administration of doses of enteramine picrate larger than 1 mg. per kilogram of body weight regularly provokes a pressure fall, accompanied with intense cutaneous vasodilatation (reddening and itching). Doses smaller than 1 mg. per kilogram of body weight have no effect on the blood pressure.

Observations in man are as yet few; it seems, however, that with large doses moderate pressor rises may take place.

From the above experimental data the conclusion must be drawn that enteramine (serotonin) is not a pure pressor substance, such as epinephrine or arterenol. Rather it is a principle which may cause hypertensive, hypotensive, or biphasic

13. Erspamer, V.: Pharmakologische Studien über Enteramin: Über die Wirkung von Acetonextrakten der Kaninchenmagenschleimhaut auf den Blutdruck und auf isolierte überlebende Organe, Arch. exper. Path. u. Pharmakol. **196**:343, 1940.

responses, according to the animal species, the dose, and the condition of the circulatory system. The problem of the existence of a true tachyphylaxis for enteramine deserves further consideration.

B. *Enteramine and 1-Hydrazinophthalazine.*—1. Blood Pressure: 1-Hydrazinophthalazine in doses of more than 1 mg. per kilogram of body weight, administered intravenously in the dog, causes a remarkable and prolonged fall of the blood

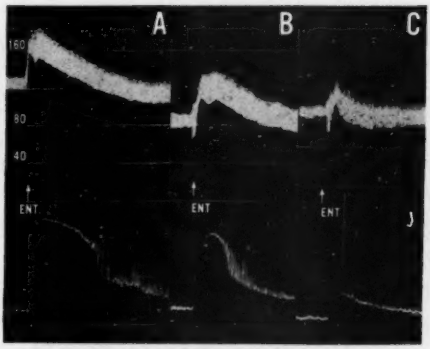

Fig. 3.—Readings on a dog weighing 10 kg., anesthetized with pentobarbital. From top to bottom: blood pressure, movements of urinary bladder *in situ*, time in minutes. Enteramine picrate (*ENT*), 1,000 γ, was given intravenously. Between *A* and *B*, 1 mg. of 1-hydrazinophthalazine per kilogram of body weight was injected intravenously; between *B* and *C*, another 1 mg. of the drug per kilogram of body weight was given. Pressor response to enteramine is reduced but not abolished; also reaction of urinary bladder is conspicuously weakened.

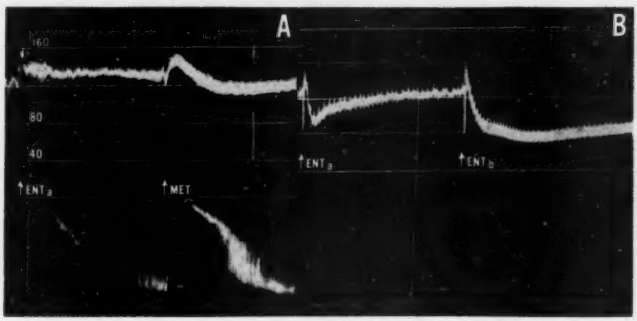

Fig. 4.—Readings on a dog weighing 6 kg., anesthetized with pentobarbital. From top to bottom: blood pressure, movements of urinary bladder *in situ*, time in minutes. Administration of 400 γ of enteramine picrate is indicated by ENT_a; that of 2,000 γ of enteramine picrate, by ENT_b, and that of 1,000 γ of 5-methoxytryptamine hydrochloride intravenously, by *MET*. Between *A* and *B*, 2 mg. of 1-hydrazinophthalazine per kilogram of body weight was given intravenously. After administration of 1-hydrazinophthalazine, the pressor response to enteramine is clearly reversed to depressor; the reaction of the urinary bladder is also strongly reduced.

pressure. If enteramine is injected during the hypotensive phase, the response to the substance may remain hypertensive but attenuated, or it may become biphasic, as observed by Taylor and associates, or even decidedly hypotensive.

It appears that the Cleveland researchers consider the drug 1-hydrazinophthalazine as a nearly specific antiserotonin agent. We do not agree with this point

of view. We believe simply that, owing to the particular conditions of depression of the smooth vascular muscle provoked by 1-hydrazinophthalazine, the potential hypotensive action of enteramine is revealed more easily than its moderate and irregular hypertensive action.

It must not be forgotten that the pressor effects of epinephrine and arterenol are also greatly depressed by 1-hydrazinophthalazine. This fact, already ascertained by Taylor and co-workers, was fully confirmed by us. We must also remember that the alleged antiserotonin action of 1-hydrazinophthalazine is by no means peculiar to this drug; after injection of crude salivary extracts of Eledone (a polyp), which contain a strong hypotensive principle, the pressor response to enteramine may alter in the same manner.

Finally, it must be pointed out that while, after administration of 1-hydrazinophthalazine, serotonin, as a rule, causes a sustained fall of the blood pressure, occasionally following an initial slight rise, this fall has never been observed with the cerebral pressor substance. The hypertensive action of this substance is only lessened and finally abolished, but never inverted, by 1-hydrazinophthalazine.

Fig. 5.—Readings on isolated estrous uterus of a rat suspended in 10 ml. of Tyrode's solution (a solution containing sodium chloride, potassium chloride, calcium chloride, magnesium chloride, monosodium phosphate, sodium bicarbonate, glucose, and water). *E* indicates administration of 0.5 γ of enteramine picrate; C_1, that of 100 γ of 1-hydrazinophthalazine, and C_2, that of 500 γ of 1-hydrazinophthalazine. 1-Hydrazinophthalazine does not appreciably reduce the uterus-stimulating action of enteramine.

2. Estrous Uterus of the Rat: This organ is one of the best biological test objects for enteramine. It responds to the substance with an augmentation in the amplitude and frequency of the movements and with an increase in tonus.

Ten to 500 γ of 1-hydrazinophthalazine does not at all modify the action of 1 γ of enteramine picrate; 1,000 to 2,000 γ slightly lessens it, but does not abolish it.

3. Diuresis of Hydrated Rats: In doses of 0.1 to 0.5 mg. per kilogram of body weight, injected subcutaneously, 1-hydrazinophthalazine seems to increase the diuresis of hydrated rats, owing probably to its renal vasodilator properties; in doses of 1 mg. per kilogram of body weight it leaves practically unaltered the urine flow; in doses of 2 mg. per kilogram of body weight and above, 1-hydrazinophthalazine clearly inhibits the water diuresis (decrease of the intraglomerular hydrostatic pressure, consequent to the fall of the systemic blood pressure). More exact investigations on this subject are in progress.

In experiments carried out on 100 groups of four rats each, the administration of 0.1, 0.5, and 1 mg. of 1-hydrazinophthalazine per kilogram of body weight failed to modify appreciably the antidiuretic action caused by 0.1, 0.3, 1, and 2 mg. of enteramine picrate per kilogram of body weight.

C. *Enteramine, Ganglion-Blocking Drugs, and Sympatholytic Agents.*—The antidiuretic action of 0.2 to 1 mg. of enteramine picrate per kilogram of body weight is left unaltered, or nearly so, by priscoline® (2-benzyl-2-imidazoline) hydrochloride, 5 to 10 mg. per kilogram of body weight; piperoxan, 10 to 20 mg. per kilogram of body weight; "prosympal" (diethylaminomethyl-1, 4-benzodioxan), 10 to 20 mg. per kilogram of body weight, and yohimbine, 1 to 3 mg. per kilogram of body weight, given intraperitoneally. It is, on the contrary, strongly inhibited or even abolished by dihydroergotamine, 1 to 2 mg. per kilogram of body weight, and dibenamine® (dibenzyl-β-chloroethylamine) hydrochloride, 5 to 20 mg. per kilogram of body weight, when these substances are administered intraperitoneally one to three hours before the injection of enteramine.[14]

Small doses of ganglion-blocking agents (1 to 2 mg. per kilogram of body weight of hexamethonium or pentamethonium, subcutaneously) are ineffective; in larger doses they enhance the enteramine antidiuresis, presumably on account of their systemic hypotensive action, which causes a fall of the intraglomerular filtration pressure.

Tetraethylammonium chloride given intravenously in the dog at a dosage of 30 mg. per kilogram of body weight has the effect of greatly augmenting the pressor action of epinephrine. The pressor responses to enteramine, to 5-methoxytryptamine, and to tryptamine are similarly augmented, but to considerably less degree.

Dibenamine® (10 to 20 mg. per kilogram of body weight) and yohimbine (1 mg. per kilogram of body weight) are able to diminish, but never to abolish or to reverse, the pressor response to enteramine.

The ganglion-blocking drugs are practically ineffective on the enteramine contraction of the estrous uterus of the rat; the same may be said for piperoxan, "prosympal," and priscoline.® Dihydroergotamine, on the contrary, and with a much clearer reaction dibenamine® strongly and irreversibly inhibit the response of the isolated organ even to enormous doses of enteramine and other indolalkylamines.

With the estrous uterus and the diuresis of the rat as test objects, dibenamine® and dihydroergotamine may be considered as powerful, true antienteramine agents. Furthermore, the estrous uterus of the rat, assayed before and after dibenamine® administration, appears to be the most specific and sensitive test object we have presently at our disposal for the biological estimation of enteramine.

COMMENT

The above-reported experiments and observations do not agree with the hypothesis held by Taylor, Page, and Corcoran on the enteramine- (serotonin)-like nature of their cerebral vasopressor principle.

Serotonin (enteramine) is by no means a pure hypertensive substance, inasmuch as it may possess, according to circumstances, hypertensive, hypotensive, and biphasic actions.

The drug 1-hydrazinophthalazine is, in its turn, in no way a specific antienteramine agent. High concentrations of 1-hydrazinophthalazine do not abolish either the uterus-stimulating or the antidiuretic action of enteramine.

14. Erspamer, V.: Modificazioni delle azioni dell'enteramina ad opera di farmaci simpaticomimetici e di farmaci simpaticolitici, Ricerca scient. e ricostruz., to be published.

It is certainly true that 1-hydrazinophthalazine, injected into the dog, may lessen, abolish, or even invert the pressor action of enteramine, but it appears that this property simply represents a nonspecific consequence of the depressive action of 1-hydrazinophthalazine on the tonus and the reactivity of the smooth vascular muscle. The hypertensive action of arterenol and epinephrine is also lessened by 1-hydrazinophthalazine, and, on the other hand, other hypotensive substances are capable of diminishing the pressor response to enteramine.

Hence, 1-hydrazinophthalazine cannot be considered a pharmacological tool for the differentiation of active substances.

Additional, stronger evidence is required to make the hypothesis of the Cleveland researchers acceptable.

Enteramine possesses a remarkable stimulant action on the urinary bladder of the dog *in situ*, as well as a powerful action on the intrarenal vascular bed. It would be interesting to see whether the cerebral vasopressor principle also causes an increase of the tonus and the motility of this organ and whether this principle does modify the intrarenal blood flow.

SUMMARY AND CONCLUSIONS

Serotonin, as described by Rapport and co-workers, is circulating enteramine.

This substance is by no means a pure hypertensive agent, inasmuch as it can provoke hypertensive, hypotensive, and biphasic reactions, according to the animal species, the dose, and the conditions of the circulatory system.

1-Hydrazinophthalazine (C-5968) cannot be considered a specific inhibitor of enteramine.

So far, no convincing evidence exists that the cerebral vasopressor principle of Taylor, Page, and Corcoran belongs to the series of the enteramine-like substances. Therefore, all the clinical and etiopathogenetic deductions which the authors have drawn from their hypothesis are premature.

ADDENDUM

After this article had been submitted for publication, two papers on serotonin appeared. Page,[15] in the one, and Freyburger and associates,[16] in the other, fully confirm those erratic effects of enteramine on the blood pressure to which particular attention was paid in this communication.

15. Page, I. H.: The Vascular Action of Natural Serotonin, 5- and 7-Hydroxytryptamine and Tryptamine, J. Pharmacol. & Exper. Therap. **105**:58, 1952.

16. Freyburger, W. A.; Graham, B. E.; Rapport, M. M.; Seay, P. H.; Govier, W. M.; Swoap, O. F., and Vander Brook, M. J.: The Pharmacology of 5-Hydroxytryptamine (Serotonin), J. Pharmacol. & Exper. Therap. **105**:80, 1952.

Progress in Internal Medicine

RECENT ADVANCES IN NUTRITION AND METABOLISM

Review of the Literature, 1951

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WALTER G. UNGLAUB, M.D.
AND
JANIS GIBBENS, M.S.
NEW ORLEANS

PROBLEMS of nutrition and metabolism are being investigated so widely and from such diverse angles that only a few facets of the mass of increasing knowledge can be presented in a brief review. During the past year, studies of the antianemia vitamins, of nutritive interrelationships, and of fat metabolism and the role of nutrition in the pathogenesis of atherosclerosis were among the most important to the practicing physician.

CALORIC NUTRITION

After a lapse of many years, caloric nutrition is again receiving the attention it justly deserves. Obesity is now recognized as probably the most important form of malnutrition in the United States, while caloric undernutrition is known to be the predominant problem in many other countries of the world. The hazards of obesity have been well documented by Armstrong,[1] and the management of the obese patient has been presented in detail in several publications.[2] A new method for promoting weight reduction, with a group approach furnishing psychological motivation, appears to have interesting possibilities which should be explored further.[3] Leverton and Gram[4] studied calcium, phosphorus, and nitrogen metabolism during weight reduction in young women and pointed out the possibilities of nutritional impairment with reducing regimens.

The prevalence of undernutrition in many parts of the world, the decrease in net available food supply per capita, and the maldistribution of food supplies have

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Dr. Goldsmith is Professor of Medicine, Dr. Unglaub, Assistant Professor of Medicine, and Miss Gibbens, Nutritionist.

1. Armstrong, D. B.; Dublin, L. I.; Wheatley, G. M., and Marks, H. H.: Obesity and Its Relation to Health and Disease, J. A. M. A. **147**:1007, 1951. Armstrong, D. B.; Dublin, L. I.; Bannett, E. C., and Marks, H. H.: Influence of Overweight on Health and Disease, Postgrad. Med. **10**:407, 1951.

2. Jolliffe, N., and Alpert, E.: "Performance Index" as a Method for Estimating Effectiveness of Reducing Regimens, Postgrad. Med. **9**:106, 1951. Barborka, C. J.: Present Status of Obesity Problem, J. A. M. A. **147**:1015, 1951. Barach, J. H.: Obesity: Its Associated Diseases and Treatment, Am. J. Digest. Dis. **19**:37, 1952. Weiner, S.: The Treatment of Obesity, ibid. **19**:24, 1952. Jolliffe, N.: Reduce and Stay Reduced, New York, Simon & Schuster, Inc., 1952.

3. Chapman, A. L.: Weight Control: A Simplified Concept, Pub. Health Rep. **66**:725, 1951.

4. Leverton, R. M., and Gram, M. R.: Further Studies of Obese Young Women During Weight Reduction: Calcium, Phosphorus and Nitrogen Metabolism, J. Am. Dietet. A. **27**:480, 1951.

stimulated renewed interest in man's caloric requirements.[5] The Food and Agriculture Organization of the United Nations established an International Committee on Calorie Requirements whose report outlines principles and recommendations for estimating the caloric needs of populations which vary in body size, age composition, and environmental temperature.[6] This report should be of great value to public health authorities and departments of agriculture and should stimulate research leading to more accurate determination of caloric needs. At present, data on the energy expenditure in both occupational and nonoccupational activity are meager. A recent study by Lehmann and associates,[7] who made 1,800 determinations of energy expenditure by industrial workers engaged in 167 different activities, is a valuable contribution. Important studies of the energy requirement of boys and girls in various types of activities have been carried out by investigators at the Nutrition Laboratory, Teachers College, Columbia University, in cooperation with the Bureau of Human Nutrition and Home Economics of the United States Department of Agriculture.[8] Macy and Hunscher[9] have emphasized the importance of calories during growth and pointed out that as few as 10 calories per kilogram of body weight per day may make the difference between progress and failure.

Clinical evaluation of caloric nutrition is inexact. In most instances findings for an individual subject are compared with values in some type of height-weight table. Unfortunately, these standards usually represent arithmetical averages, often of selected groups, and do not take into consideration the relative proportion in the total mass of several body components: muscles, adipose tissue, bones, and extracellular fluid. Brozek and Keys[10] have recently reviewed quantitative methods for evaluation of leanness and fatness in man. The most promising indirect method is measurement of the thickness of skin folds with specially constructed calipers. Other methods include estimation of total excess fat from the specific gravity of the body, estimation of body fat from the body water content, and determination of the thickness of the subcutaneous tissues from roentgenograms.

Adaptation to caloric restriction has been studied in the Laboratory of Physiological Hygiene at the University of Minnesota,[11] and a book reviewing knowledge of the biology of human starvation has been published by Keys.[12]

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5. Man's Calorie Requirements, Nutrition Rev. **9**:40, 1951.

6. Calorie Requirements, Report of Committee on Calorie Requirements, United Nations Food and Agriculture Organization, Nutritional Studies No. 5, New York, Columbia University Press, 1950.

7. Lehmann, G.; Müller, E. A., and Spitzer, H.: Der Calorienbedarf bei gewerblicher Arbeit, Arbeitsphysiologie, **14**:166, 1949-1950.

8. Thompson, E. M.; Bal, M. E. R.; McIntosh, E. M.; MacLeod, G., and Taylor, C. M.: The Energy Expenditure for Quiet Play and Cycling of Girls 6 to 14 Years of Age, J. Nutrition **44**:275, 1951. Taylor, C. M.; Pye, O. F.; Schafer, M., and Wing, S.: The Energy Expenditure of Boys and Girls 9 to 11 Years of Age (1) Washing and Wiping Dishes, (2) Boys Engaged in Carpentry, and (3) Girls Sewing, ibid. **44**:295, 1951.

9. Macy, I. G., and Hunscher, H. A.: Calories: A Limiting Factor in the Growth of Children, J. Nutrition **45**:189, 1951.

10. Brozek, J., and Keys, A.: Evaluation of Leanness-Fatness in Man: A Survey of Methods, Nutrition Abstr. & Rev. **20**:247, 1950; The Evaluation of Leanness-Fatness in Man: Norms and Interrelationships, Brit. J. Nutrition **5**:194, 1951.

11. Taylor, H. L., and Keys, A.: Adaptation to Caloric Restriction, Science **112**:215, 1950.

12. Keys, A.; Brozek, J.; Henschel, A.; Mickelsen, O., and Taylor, H. L.: The Biology of Human Starvation, Minneapolis, University of Minnesota Press, 1950, Vols. 1 and 2.

Calories assume a most important position in providing adequate nutrition for the patient who must be fed parenterally. Calories are essential for economical utilization of parenterally administered amino acids and for maintenance of positive nitrogen balance.[13] Alcohol given intravenously in 5 to 10% solution is a useful source of calories in addition to that furnished by dextrose and amino acids.[14] The use of fat emulsions in parenteral nutrition is discussed in the section dealing with fat metabolism.

FAT METABOLISM

Relationships of dietary fat, cholesterol, and blood lipids to the pathogenesis of atherosclerosis were studied extensively during the past year. In a recent symposium, Katz[15] presented an excellent review of factors involved in the experimental production of atherosclerosis in animals. He pointed out that atherosclerosis had not been produced except by cholesterol administration or, in avian species, by use of diethyl stilbestrol, which stimulates endogenous hypercholesteremia. Lesions which developed in chicks were reversible to some extent, and an adequate diet contributed to prevention. Choline and inositol had no effect on induced atherosclerosis in several species. Administration of thyroid extract decreased, and that of salt and adrenal steroids increased, the severity of atherosclerosis under certain conditions. Since cholesterol has an enterohepatic circulation, an attempt was made to reduce the levels of cholesterol in the blood of chicks by oral administration of aluminum hydroxide, with some success in preliminary experiments. Biggs and Kritchevsky[16] studied experimental atherosclerosis in chicks with the use of tritium-labeled cholesterol and found that exogenous cholesterol formed the bulk of the cholesterol in atheromatous deposits. Cholesterol synthesis continued even after massive prolonged oral administration.

Findings in animal studies cannot necessarily be applied to man. The diets used in inducing experimental atherosclerosis contain much more cholesterol than is found in human diets. Moreover, Keys[17] has shown that levels of cholesterol in the blood in man appear to be independent of dietary intake in the range of 0 to 700 mg. daily. On the other hand, serum cholesterol levels appear to be markedly influenced by the fat content of the diet. Low levels are found in persons receiving a rice-fruit diet in the treatment of hypertension.[18] Keys[17] found a decrease in serum cholesterol when the diet was devoid of fat and a large increase when fat was added to the regimen. It is known that man has a large capacity for synthesis of cholesterol from acetate; 1.5 to 2 gm. may be formed daily.[19]

13. Rice, C. O.; Orr, B.; Treloar, A. E., and Strickler, J. H.: Parenteral Nutrition in Surgery: Significance of Calories and Protein in Maintaining a Positive Nitrogen Balance, A. M. A. Arch. Surg. **61**:977, 1951.

14. Rice and others.[13] Karp, M., and Sokol, J. K.: Intravenous Use of Alcohol in the Surgical Patient, J. A. M. A. **146**:21, 1951.

15. Katz, L. M.: Experimental Atherosclerosis, Circulation **5**:101, 1952.

16. Biggs, M. W., and Kritchevsky, D.: Observations with Radioactive Hydrogen (H^3) in Experimental Atherosclerosis, Circulation **4**:34, 1951.

17. Keys, A.: Human Atherosclerosis and the Diet, Circulation **5**:115, 1952.

18. Starke, H.: Effect of the Rice Diet on the Serum Cholesterol Fractions of 154 Patients with Hypertensive Vascular Disease, Am. J. Med. **9**:494, 1950.

19. Gould, R. G.: Lipid Metabolism and Atherosclerosis, Am. J. Med. **11**:209, 1951.

Evidence relating cholesterol to the development of atherosclerosis in man includes (1) a high incidence of early and severe atherosclerosis in persons with diseases associated with hypercholesteremia, such as diabetes mellitus and nephrosis; (2) a tendency for high and variable serum cholesterol levels in association with coronary artery disease,[20] and (3) some parallelism between the increase with age in cholesterol concentration in the blood and the incidence of atherosclerosis. McMahon,[21] in a study of cholesterol levels of 822 persons, found an increase in mean values with advancing years, with a decline in later decades. Gertler and associates [22] noted an increase in phospholipid as well as in cholesterol levels with advancing years in health but a smaller increase in phospholipid concentration with age in coronary artery disease. They suggested that the levels of cholesterol and phospholipid in the serum are less important than the ratio of these lipids.

Variations in the concentration of several blood lipids were studied in normal persons by Goldbloom [23] and by Russ and associates.[24] The latter investigators presented evidence that essentially all the cholesterol and phospholipid of plasma is combined with protein in the form of either alpha or beta lipoprotein. A photometric method for determination of total and free cholesterol and of the cholesterol-ester ratio was reported.[25] The effect of diets of varying fat content on the amount and types of fatty acids and other lipids in plasma and feces was reported by Wollaeger.[26] Serum lipids were determined in lipemia retinalis,[27] in diabetes mellitus,[28] and after ingestion of butterfat with and without polysorbate 80 ("tween 80").[29]

Adlersberg [30] postulated from a genetic analysis of families with xanthomatosis and families of patients with coronary artery disease that early coronary athero-

20. Gertler, M. M.; Garn, S. M., and Lerman, J.: The Interrelationships of Serum Cholesterol, Cholesterol Esters and Phospholipids in Health and in Coronary Artery Disease, Circulation **2**:205, 1950.

21. McMahon, A.; Allen, H. N.; Weber, C. J., and Missey, W. C., Jr.: Hypercholesterolemia, South. M. J. **44**:993, 1951.

22. Gertler and others.[20] Gertler, M. M.; Garn, S. M., and Bland, E. F.: Age, Serum Cholesterol and Coronary Artery Disease, Circulation **2**:517, 1950.

23. Goldbloom, A. A.: Clinical Studies of Blood Lipid Metabolism: I. Normal Blood Lipid Variations of Phospholipids, Neutral Fats, Total Lipids and Lipid Fraction Percentages, Am. J. Digest. Dis. **19**:9, 1952.

24. Russ, E. M.; Eder, H. A., and Barr, D. P.: Protein-Lipid Relationships in Human Plasma: I. In Normal Individuals, Am. J. Med. **11**:468, 1951.

25. Saifer, A.: Photometric Determination of Total and Free Cholesterol and the Cholesterol Ester Ratio of Serum by a Modified Liebermann-Burchard Reaction, Am. J. Clin. Path. **21**:24, 1951.

26. Wollaeger, E. E.; Lundberg, W. O.; Chipault, J. R., and Mason, H. L.: Fecal Fatty Acids and Other Lipids: A Study of 2 Normal Human Adults Taking (1) a Diet Free of Lipid, and (2) a Diet Containing Triolein as the Only Lipid, J. Clin. Invest. **29**:853, 1950.

27. Carfagno, S. C., and Steiger, W. A.: The Serum Lipids in Lipemia Retinalis, Am. J. M. Sc. **221**:379, 1951.

28. Hinch, E. F., and Carbonaro, L.: The Serum Esterified Fatty Acids with Fat Tolerance Tests in Diabetes Mellitus, J. Lab. & Clin. Med. **36**:835, 1950.

29. Brien, F. S.; Turner, D. A.; Watson, E. M., and Geddes, J. H.: A Study of Carbohydrate and Fat Absorption from the Normal and Diseased Intestine in Man: II. Changes in the Serum Lipids in Man After the Ingestion of Butterfat With and Without Tween 80 (Sorlate), Gastroenterology **20**:294, 1952.

30. Adlersberg, D.: Hypercholesteremia with Predisposition to Atherosclerosis, Am. J. Med. **11**:600, 1951.

sclerosis may be a hereditary disturbance of lipid metabolism manifested by elevated cholesterol levels and transmitted as a dominant trait. Gertler and co-workers [31] pointed out a relation between body morphology and serum lipids and suggested a method of selecting persons under 40 years of age who might be prone to myocardial infarction.[32]

Chylomicrons, the large fat particles found in the blood after a fat meal, have been implicated in the pathogenesis of atherosclerosis. Necheles [33] reviewed previous work on this subject and reported additional findings. Chylomicronemia tends to be greater and of longer duration in old than in young subjects. A lipase preparation depressed chylomicronemia in the aged to approximately the levels found in young men. Zinn and Griffith [34] found a significant qualitative difference in the physical size of lipid particles between atherosclerotic and nonatherosclerotic subjects. Frailing and Owen [35] described a simple method of counting chylomicrons in whole blood.

Evans [36] reported an ingenious experiment in which oxylated blood from normal persons and from persons with disease (myocardial infarction, vascular disease, and diabetes mellitus) was pulsated for 72 hours against strips of normal aorta obtained at autopsy. Examination of the strips in unstained sections with polarized-light microscopy showed deposition of birefringent particulate material in the walls in 75% of tests with blood from persons with disease and in only 5% of tests with blood from normal persons. The microscopic changes resembled early atheromas.

Studies of lipoproteins in human serum with an ultracentrifugal flotation technique were continued by Gofman and associates.[37] Groups of molecules in serum are classified according to the rate at which they migrate in terms of Svedberg flotation (S_f) units. A strong correlation between the concentration of S_f 12-20 molecules and atherosclerosis and a less marked correlation between the level of S_f 35-100 lipoproteins and atherosclerosis were reported. Diet influenced the concentration of S_f 35-100 molecules. The concentration of S_f 12-20 molecules is higher than normal in patients with diabetes mellitus, hypothyroidism, hypertension, nephrosis, and myocardial infarction. The recurrence rate of myocardial infarction was 20% yearly when levels of S_f 12-20 lipoproteins were 100 mg. per 100 cc., as compared with a rate of 6% yearly when levels were 50 mg. per 100 cc. Restriction of dietary fat and cholesterol depressed S_f 12-20 levels and significantly

31. Gertler, M. M.; Garn, S. M., and Sprague, H. B.: Cholesterol, Cholesterol Esters and Phospholipids in Health and in Coronary Artery Disease: II. Morphology and Serum Lipids in Man, Circulation **2**:380, 1950.

32. Gertler, M. M.; Garn, S. M., and White, P. D.: Young Candidates for Coronary Heart Disease, J. A. M. A. **147**:621, 1951.

33. Necheles, H.: The Phenomenon of Chylomicrons in Fat Absorption and Atherosclerosis, Am. J. Digest. Dis. **18**:229, 1951.

34. Zinn, W. J., and Griffith, G. C.: A Study of Serum Fat Globules in Atherosclerotic and Non-Atherosclerotic Male Subjects, Am. J. M. Sc. **220**:597, 1950.

35. Frailing, J. M., and Owen, C. I.: Chylomicrons of Blood: A Résumé of Literature and a Recommended Counting Procedure, Am. J. Clin. Path. **21**:508, 1951.

36. Evans, S. M.; Ihrig, H. K.; Means, J. A.; Zeit, W., and Haushalter, E. E.: Atherosclerosis: An in Vitro Demonstration of Phenomena Resembling the Early Lesions of Atherosclerosis, J. Lab. & Clin. Med. **38**:807, 1951.

37. Gofman, J. W.; Jones, H. B.; Lyon, T. P.; Lindgren, F.; Strisower, B.; Colman, D., and Herring, V.: Blood Lipids and Human Atherosclerosis, Circulation **5**:119, 1952.

decreased the recurrence of coronary occlusion. Jones and associates [38] reported that administration of heparin suppressed development of atherosclerosis in cholesterol-fed rabbits and produced changes in lipoprotein patterns in human sera. Relief of anginal pain was noted in patients who received heparin in doses of 25 or 100 mg. once or twice weekly. Deficiency of heparin or a heparin-like substance was suggested as a possible causative factor in disturbances of lipid metabolism. Block and associates [39] found that administration of heparin following a standard fat meal increased serum translucence to a greater extent in normal persons than in atherosclerotic subjects.

Jones and associates [38] believe that the correlation of S_f 12-20 lipoprotein levels with atherosclerosis is much greater than the correlation of serum cholesterol levels with this condition and that determination of the concentration of S_f 12-20 molecules is valuable in predictive segregation of atherosclerotic from normal persons. Contrary to this, Keys [40] presented data indicating that the concentration of S_f 12-20 molecules is no more critical than that of total cholesterol in serum in predicting or detecting coronary artery disease.

Serum lipoprotein levels have been determined in normal children,[41] in children with hepatitis,[42] and in those with juvenile diabetes.[43] The plasma lipoprotein molecule has been studied by electron micrography.[44] A number of reviews dealing with lipid metabolism and atherosclerosis have been published in addition to those cited above.[45] Obviously, much more investigation is essential for delineation of the role of cholesterol, S_f 12-20 lipoproteins, and chylomicrons in the pathogenesis of atherosclerosis. There is little evidence that removing cholesterol from the diet is advantageous in the prevention or treatment of coronary artery disease.[46] It has been demonstrated that a low-fat diet or a low-fat, low-cholesterol diet will

38. Jones, H. B.; Gofman, J. W.; Lindgren, F. T.; Lyon, T. P.; Graham, D. M.; Strisower, B., and Nichols, A. V.: Seminars on Arteriosclerosis: Lipoproteins in Atherosclerosis, Am. J. Med. **11**:358, 1951.

39. Block, W. J., Jr.; Mann, F. D., and Barker, N. W.: Effect of Small Doses of Heparin in Increasing the Translucence of Plasma During Alimentary Lipemia: Studies in Normal Individuals and Patients with Atherosclerosis, Proc. Staff Meet., Mayo Clin. **26**:246, 1951.

40. Keys, A.: "Giant Molecules" and Cholesterol in Relation to Atherosclerosis, Bull. Johns Hopkins Hosp. **88**:473, 1951; Cholesterol, "Giant Molecules" and Atherosclerosis, J. A. M. A. **147**:1514, 1951.

41. Kempe, C. H.; Silver, H. K.; Smyth, F. S.; Gofman, J. W., and Jones, H. B.: The Lipoproteins of Serum in Infancy and Childhood: I. Lipoproteins in Normal Children, J. Pediat. **40**:11, 1952.

42. Pierce, F. T., and Gofman, J. W.: Lipoproteins, Liver Disease, and Atherosclerosis, Circulation **4**:25, 1951.

43. Carr, J. H.; Kempe, C. H.; Silver, H. K.; Smyth, F. S.; Gofman, J. W., and Jones, H. B.: The Lipoproteins of Serum in Infancy and Childhood: II. Lipoprotein Levels in Juvenile Diabetes Mellitus, J. Pediat. **40**:19, 1952.

44. Prendergast, J. J., and Teague, D. M.: Electron Micrograph of Plasma Lipoprotein Molecule (S_f 10-20), Circulation **4**:23, 1951.

45. (*a*) Gould. [19]. (*b*) Duff, G. L., and McMillan, G. C.: Pathology of Atherosclerosis, Am. J. Med. **11**:92, 1951. (*c*) Sacks, M. S.: Lipids and Atherosclerosis, Ann. Int. Med. **35**:938, 1951. (*d*) Plotz, M. B.: Fat Metabolism in Coronary Atheroma, Postgrad. Med. **10**:459, 1951. (*e*) Davidson, J. D.: Diet and Lipotropic Agents in Arteriosclerosis, Am. J. Med. **11**:736, 1951.

46. Keys.[17] Davidson.[45e] Gertler, M. M.; Garn, S. M., and White, P. D.: Diet, Serum Cholesterol and Coronary Artery Disease, Circulation **2**:696, 1950.

lead to a decrease in serum cholesterol in certain circumstances,[47] and that a low-fat, low-cholesterol diet may lead to a decrease in S_f 12-20 lipoprotein molecules.[37] In light of present knowledge, the only practical dietary advice in coronary artery disease would seem to be avoidance of high-fat diets and of excessive caloric intake. Choline and inositol have not been demonstrated to have a specific influence on atherosclerosis or serum cholesterol levels in man.[45d]

The potential importance of preparations of fat for parenteral use as a source of calories is well recognized. Unfortunately, most of the fat emulsions developed thus far have produced febrile reactions. Lambert and associates [48] were unable to prepare emulsions which passed the U. S. P. XIII pyrogen test and suggested that thermogenicity might be an inherent property of fat emulsions. Meng [49] found that emulsions which produced little febrile reaction when first prepared developed pyrogenicity after the lapse of several months. Neptune and co-workers [50] administered 64 infusions of 1 liter or more of 15% fat emulsion to a series of nine patients without encountering rises in temperature.

Murray and Freeman [51] studied the morphologic distribution of intravenously injected fatty chyle and artificial fat emulsion in rats and dogs and found that artificial olive oil emulsion seemed to be handled as a foreign material rather than as natural fat. Fat emulsions for oral use have been prepared and found to be well assimilated in amounts providing 1,000 to 2,200 calories daily.[52]

NUTRITION AND HYPERTENSION

The effectiveness of the rice-fruit diet and of other low-salt regimens in the treatment of hypertension remains controversial in spite of extensive investigation. Chasis,[53] Weston,[54] and their collaborators, who studied 12 and 9 persons, respectively, doubt the efficacy of the rice diet in reducing blood pressure. Chasis [53] noted a decrease in glomerular filtration rate, renal plasma flow, and maximal tubular excretory capacity in the majority of patients during the rice regimen. Tubular excretion returned to control values when an adequate diet was instituted. Wes-

47. Keys.[17] Davidson.[45e] Priddle, W. W.: Hypercholesteremia: An Analysis of 529 Cases and Treatment of 297 by a Low Animal Fat Diet and Desiccated Thyroid Substance, Ann. Int. Med. **35**:836, 1951.

48. Lambert, G. F.; Miller, J. P., and Frost, D. V.: Febrile Response Following Intravenous Administration of Fat, Am. J. Physiol. **164**:490, 1951.

49. Meng, H. C.: A Fat Emulsion for Intravenous Nutrition in Rabbits, J. Lab. & Clin. Med. **37**:222, 1951.

50. Neptune, E. M., Jr.; Geyer, R. P.; Saslaw, I. M., and Stare, F. J.: Parenteral Nutrition: XII. The Successful Intravenous Administration of Large Quantities of Fat Emulsion to Man, Surg., Gynec. & Obst. **92**:365, 1951.

51. Murray, R. G., and Freeman, S.: The Morphologic Distribution of Intravenously Injected Fatty Chyle and Artificial Fat Emulsion in Rats and Dogs, J. Lab. & Clin. Med. **38**:56, 1951.

52. Van Itallie, T.; Logan, W. B.; Smythe, R. L.; Geyer, R. P., and Stare, F. J.: Fat Emulsions for Oral Nutrition: IV. Metabolic Studies on Human Subjects, Metabolism **1**:80, 1952.

53. Chasis, H.; Goldring, W.; Breed, E. S.; Schreiner, G. E., and Bolomey, A. A.: Salt and Protein Restriction: Effects on Blood Pressure and Renal Hemodynamics in Hypertensive Patients, J. A. M. A. **142**:711, 1950.

54. Weston, R. E.; Hellman, L.; Escher, D. J. W.; Edelman, I. S.; Grossman, J., and Leiter, L.: Studies on the Influence of the Low Sodium Cardiac Diet and the Kempner Regimen on Renal Hemodynamics and Electrolyte Excretion in Hypertensive Subjects, J. Clin. Invest. **29**:639, 1950.

ton,[54] using similar renal-function studies, found a decrease in only the glomerular filtration rate, although after administration of mercurial diuretics renal plasma flow decreased. A fall in plasma volume occurred in five of nine patients. Murphy [55] found a decrease in plasma volume, in extravascular thiocyanate space, and in total circulating plasma protein in patients receiving the rice diet. Myers and Murphy [56] reported a decrease in sulfobromophthalein (bromsulphalein®) sodium clearance with normal hepatic blood flow and splanchnic oxygen consumption in such subjects. Dole and associates [57] noted a significant reduction in mean blood pressure and a decrease in heart size, improvement in the optic fundi, and a return of electrocardiographic findings toward normal in five of six patients with hypertension treated with the rice diet. Restriction of sodium but not of chloride appeared to be necessary for the clinical effect. The five subjects with normal renal function approached equilibrium with respect to nitrogen, sodium, potassium, chloride, and body weight after three or more months. Clinical benefit appeared to be associated with successful adaptation to the limitation of sodium intake. Watkin and associates [58] in a well-controlled study of 50 patients with essential hypertension found that the percentage of patients showing a reduction in blood pressure, decrease in heart size, and improvement in retinopathy and in symptomatology was far higher when the rice diet was administered than during the control period. Addition of sodium chloride to the rice diet, by alternation with a placebo capsule, resulted in elevation of the blood pressure in most instances. Addition of salt-poor protein and fat had little effect. The authors concluded that the most significant factor in reduction of blood pressure was the low-sodium content of the diet. Currens and associates [59] observed a decrease in blood pressure in two of seven patients receiving the rice diet. The basal metabolic rate fell slightly, and renal clearance decreased. It was suggested that the latter finding may be a reflection of the low-protein content of the diet rather than an adverse effect. The negative nitrogen balance observed early in therapy diminished markedly after three to six months. Blood pressures rose when sodium chloride supplements were administered. Loofbourow and co-workers [60] concluded that in ambulatory patients the rice diet produced no better results than did conservative medical therapy. Nevertheless, in certain severely ill patients with hypertension the rice diet appeared to effect a dramatic

55. Murphy, R. J. F.: The Effect of "Rice Diet" on Plasma Volume and Extracellular Fluid Space in Hypertensive Subjects, J. Clin. Invest. **29**:912, 1950.

56. Myers, J. D., and Murphy, R. J. F.: Studies of Hepatic Function in Patients on the Rice Diet, Am. J. Med. **9**:405, 1950.

57. Dole, V. P.; Dahl, L. K.; Cotzias, G. C.; Eder, H. A., and Krebs, M. E.: Dietary Treatment of Hypertension: Clinical and Metabolic Studies of Patients on the Rice-Fruit Diet, J. Clin. Invest. **29**:1189, 1950.

58. Watkin, D. M.; Froeb, H. F.; Hatch, F. T., and Gutman, A. B.: Effects of Diet in Essential Hypertension: I. Baseline Study; Effects in 86 Cases of Prolonged Hospitalization on Regular Hospital Diet, Am. J. Med. **9**:428, 1950; II. Results with Unmodified Kempner Rice Diet in 50 Hospitalized Patients, ibid. **9**:441, 1950.

59. Currens, J. H.; Reid, E. A. S.; MacLachlan, E. A., and Simeone, F. A.: Metabolic Effects of the Rice Diet in the Treatment of Hypertension, New England J. Med. **245**:354, 1951.

60. Loofbourow, D. G.; Callahan, D., and Palmer, R. S.: The Rice Diet in Ambulatory Patients with Essential Hypertension: A 2-Year Study of 105 Patients, New England J. Med. **244**:577, 1951.

reversal of the disease process. Dole and associates [61] found that restriction of dietary sodium to 7 mEq. daily caused significant reduction in the average blood pressure of five patients with uncomplicated hypertension. This reduction was due to limitation of dietary sodium, since no changes in blood pressure were observed during a preliminary period of two to four months in which the low-sodium diet was supplemented with 180 mEq. of sodium. Losses of weight and large variations in the intake of water and protein were without significant effect on blood pressure. Upon the change from the high- to the low-sodium diet, weight loss was not proportional to sodium loss. Since the serum sodium concentration did not change significantly, the data imply an exchange of water into the cells. The authors suggested that an increase in cellular hydration may account for the beneficial effect of the low-sodium diet.

In spite of the diverse findings noted above, low-sodium diets show sufficient promise in the therapy of hypertension to justify continued exploration. It seems likely that regimens more palatable and liberal than the rice diet may be equally effective. Problems in the planning and administration of diets restricted in sodium have been discussed by Robinson.[62]

PROTEIN NUTRITION

Numerous factors which influence the utilization of protein by the animal body have recently been reviewed in an excellent symposium.[63] It is pointed out that studies of nitrogen balance express the summation of many variables which must be carefully evaluated in the interpretation of data. The total daily caloric intake, the distribution of nonprotein calories in the diet, the relation of total dietary protein to the caloric value of the ration, the time factor in feeding, the distribution of animal protein over the day's meals, the general physiologic state of the animal, and the status of body protein stores must all be considered in relation to their individual and combined effects upon nitrogen retention. A finding of interest is that the nonprotein constituents of the diet can promote growth and the sparing of body protein through mechanisms apparently unrelated to their calorigenic-sparing action. In the final interpretation of nitrogen-balance data, it must be remembered that simultaneous depletion and repletion of separate body protein compartments may not be detected, and that it is quite possible for an animal to be in positive balance even while actively depleting some labile body protein stores. Waife and his associates [64] studied the effect of protein supplementation upon nitrogen balance in 12 chronically ill subjects considered to be protein-depleted and

61. Dole, V. P.; Dahl, L. K.; Cotzias, G. C.; Dziewiatkowski, D. D., and Harris, C.: Dietary Treatment of Hypertension: II. Sodium Depletion as Related to the Therapeutic Effect, J. Clin. Invest. **30**:584, 1951.

62. Robinson, C. H.: The Restricted Sodium Diet: A Survey of Problems in Planning and Administration, J. Am. Dietet. A. **27**:1075, 1951.

63. Swanson, P. P.: Influence of Non-Protein Calories on Protein Metabolism, Federation Proc. **10**:660, 1951. Geiger, E.: Extra Caloric Function of Dietary Components in Relation to Protein Utilization, ibid. **10**:670, 1951. Allison, J. B.: Interpretation of Nitrogen Balance Data, ibid. **10**:676, 1951.

64. Waife, S. O.; Wohl, M. G., and Reinhold, J. G.: Protein Metabolism in Chronic Illness: Effect of Protein Supplementation on Nitrogen Balance, Hemoglobin, Serum Proteins, and Weight in the Malnourished and the Effect of the Nutritional Status on Nitrogen Storage, J. Lab. & Clin. Med. **36**:604, 1950.

found that the amount of nitrogen stored increased apparently in proportion to the degree of previous protein depletion. Hoffman [65] found that more nitrogen was required to produce equilibrium when a modified casein hydrolysate was the only important source of nitrogen in the diet than when the same hydrolysate was fortified with *dl*-tryptophane, *dl*-methionine, and *l*-phenylalanine in quantities which made the pattern of essential amino acids equivalent to that found by Rose [66] to constitute minimal requirements. Studies of the effect of caloric intake upon nitrogen retention in normal [67] and pregnant women [68] have emphasized the importance of an adequate supply of nonprotein calories for the proper utilization of dietary protein.

Dieckmann and his associates [69] have found a striking correlation between the condition of 302 babies, as rated by a pediatrician, and the average protein intake of the mothers during pregnancy. The incidence of spontaneous abortion in their subjects increased significantly as protein intake decreased. No relation between inadequate protein intake and toxemia of pregnancy, premature delivery, or duration of labor could be established. Studies of changes in the electrophoretic pattern of the plasma proteins in normal pregnancy and in toxemia have not established any relation between dietary protein inadequacy and the abnormalities of the serum proteins observed in the eclamptogenic syndrome.[70]

Variability of fecal nitrogen excretion found in normal subjects on constant dietary intakes indicates the need for analyses of fecal nitrogen in all nitrogen balance studies.[71] Endogenous urinary nitrogen output in adult females was found to be more closely related to urinary creatinine excretion than to weight, surface area, or basal metabolic rate.[72]

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65. Hoffman, W. S., and McNeil, G. C.: Nitrogen Requirement of Normal Men on a Diet of Protein Hydrolysate Enriched with the Limiting Essential Amino Acids, J. Nutrition **44**:123, 1951.

66. Rose, W. C.: Amino Acid Requirements of Man, Federation Proc. **8**:546, 1949.

67. Leverton, R. M.; Gram, M. R., and Chaloupka, M.: Effect of Time Factor and Calorie Level on Nitrogen Utilization of Young Women, J. Nutrition **44**:537, 1951.

68. Oldham, H., and Sheft, B. B.: Effect of Caloric Intake on Nitrogen Utilization During Pregnancy, J. Am. Dietet. A. **27**:847, 1951.

69. Dieckmann, W. J.; Turner, D. F.; Meiller, E. J.; Savage, L. J.; Hill, A. J.; Straube, M. T.; Pottinger, R. E., and Rynkiewicz, L. M.: Observations on Protein Intake and the Health of the Mother and Baby: I. Clinical and Laboratory Findings, J. Am. Dietet. A. **27**:1046, 1951. Dieckmann, W. J.; Turner, D. F.; Meiller, E. J.; Straube, M. T., and Savage, L. J.: Observations on Protein Intake and the Health of the Mother and Baby: II. Food Intake, ibid. **27**:1053, 1951.

70. Coryell, M. N.; Beach, E. F.; Robinson, A. R.; Macy, I. G., and Mack, H. C.: Metabolism of Women During the Reproductive Cycle: XVII. Changes in the Electrophoretic Patterns of Plasma Proteins Throughout the Cycle and Following Delivery, J. Clin. Invest. **29**:1559, 1950. Mack, H. C.; Robinson, A. R.; Wiseman, M. E.; Schoeb, E. J., and Macy, I. G.: Plasma Proteins in Toxemias of Pregnancy, ibid. **30**:609, 1951.

71. Toscani, V., and Whedon, G. D.: Nitrogen Loss in the Feces: The Variability of Excretion in Normal Subjects on Constant Dietary Intakes, J. Nutrition **45**:119, 1951.

72. Bricker, M. L., and Smith, J. M.: A Study of the Endogenous Nitrogen Output of College Women, with Particular Reference to Use of the Creatinine Output in the Calculation of the Biological Values of the Protein of Egg and of Sunflower Seed Meal, J. Nutrition **44**:553, 1951.

The relative value of proteins of animal and vegetable origin in human nutrition has been considered in detail in a recent symposium.[73] Although vegetable proteins are generally inferior to animal protein unless fed at high levels, they are of great potential value when used to complement a diet low in animal protein or in conjunction with concentrates which furnish animal protein factor and the necessary vitamins. Albanese and associates [74] have found that nitrogen retention and weight gain by infants fed a synthetic milk diet, in which an enzymatic digest of bovine plasma constituted the principal source of protein, were equal or superior to those obtained on an evaporated milk formula.

Williamson and his associates [75] have been unable to demonstrate any relation between wound healing in rats and the dietary intake of protein nitrogen. Their results suggest that protein sulfur in excess of that used for normal protein synthesis is an important factor in determining the rate of healing. Testosterone has not been found to alter the rate of weight loss during protein depletion in rats or the rate of weight gain during protein repletion.[76] Studies of antibody response in a group of patients with abnormal serum protein levels have shown no relation between concentration or distribution of serum protein fractions and the ability to form antibodies.[77] Rafsky [78] has found that the neutralizing action of protein preparations on gastric juice in peptic ulcer is of short duration and is followed by a gastric secretory response which produces an increase in free hydrochloric acid exceeding pretreatment levels. Protein supplements are useful in the treatment of peptic ulcer only for the correction of hypoproteinemia and not for their buffering effect. Rike and his associates [79] have reported metabolic studies in 26 patients during prolonged continuous narcosis therapy. One-half of the group was fed by gavage, the other half by intravenous administration of protein hydrolysates and dextrose. The chief effects noted were a transient decrease in serum albumin, disturbances of carbohydrate metabolism, elevation of non-protein nitrogen of the blood, and transient albuminuria. The group fed parenterally also manifested a tendency to low levels of serum cholesterol and positive cephalin flocculation tests.

73. Carpenter, K. J.: The Relative Nutritional Values of Animal and Vegetable Proteins for Animals, Brit. J. Nutrition **5**:243, 1951. Blaxter, K. L.: Conversion Factors for Vegetable and Animal Foods for Human Consumption, ibid. **5**:250, 1951. Chick, H.: Nutritive Value of Vegetable Proteins and Its Enhancement by Admixture, ibid. **5**:261, 1951. Dean, R. F. A.: The Nutritional Adequacy of a Vegetable Substitute for Milk, ibid. **5**:269, 1951.

74. Albanese, A. A.; Higgons, R. A.; MacDonald, G. E.; Felch, W. C.; Vistas, B., and Stephanson, L.: Biological Value of an Enzymatic Digest of Bovine Plasma, J. Nutrition **44**:281, 1951.

75. Williamson, M. B.; McCartney, T. H., and Fromm, H. J.: Relation of Protein Nutrition to the Healing of Experimental Wounds, Proc. Soc. Exper. Biol. & Med. **77**:302, 1951.

76. Geiger, E., and El Rawi, I.: Lack of Effect of Testosterone Propionate on Body Weight of Rats During Protein Depletion and Repletion, Metabolism **1**:145, 1952.

77. Larson, D. L., and Tomlinson, L. J.: Quantitative Antibody Studies in Man: II. The Relation of the Level of Serum Proteins to Antibody Production, J. Lab. & Clin. Med. **39**:129, 1952.

78. Rafsky, H. A.; Krieger, C. I., and Honig, L. J.: Protein Studies in Peptic Ulcer, Gastroenterology **16**:358, 1950.

79. Rike, P. M.; Loomis, E. A., Jr., and Clapp, J. S.: Metabolic Studies in Gavage and Parenteral Feeding During Prolonged Narcosis Therapy, Am. J. Digest. Dis. **18**:92, 1951.

Amino Acids.—Factors which influence the pattern of amino acid excretion have been studied by a number of investigators. Kamin and Handler [80] determined the urinary excretion of eight "endogenous" amino acids in dogs infused with various amino acids at constant rates. In almost all instances the rate of amino acid excretion was increased many times. In general, the rate of excretion of a single amino acid was most increased by the infusion of an acid of similar acidic properties. The neutral amino acids, glutamine and asparagine, were found to exert the most pronounced effect when infused. Threonine and histidine were the endogenous amino acids most readily affected. Glutamic and aspartic acid excretion did not conform to the general pattern observed. Nasset and Tully [81] studied the rate of excretion of "free" essential amino acids by human subjects on a protein-free diet. No sex differences were observed, and, with the possible exceptions of threonine and histidine, the pattern and absolute amount of amino acid excretion were little changed by adding various proteins to the diet. Sheffner and associates [82] found that, under constant conditions of nitrogen and caloric intake, larger quantities of amino acids were excreted in the urine and feces when protein hydrolysates were administered orally than when normal whole protein was given. Differences in excretion were found to be more pronounced in patients with gastric ulcer and ulcerative colitis than in normal subjects. A comprehensive study of nine subjects acutely injured by radiation revealed increased urinary amino acid excretion in three, reaching a maximum about the sixth day after exposure.[83] This was attributed to decreased ability of muscle to synthesize protein, to inhibition of amino acid oxidation in the kidneys, and to enhanced breakdown of tissue injured by radiation. Wallraff and associates [84] have determined the urinary excretion of amino acids in normal women and during pregnancy.

Plasma levels of amino acids in aged subjects have been studied by Hofstatter and associates.[85] The metabolic role of glycine has been investigated with the use of isotopically labeled amino acids by Berlin,[86] Grossman,[87] and Watson and his associates.[88] The role of isoleucine in hemoglobin synthesis in rats has been studied

80. Kamin, H., and Handler, P.: Effect of Infusion of Single Amino Acids upon Excretion of Other Amino Acids, Am. J. Physiol. **164**:654, 1951.

81. Nasset, E. S., and Tully, R. H., III: Urinary Excretion of Essential Amino Acids by Human Subjects Fed Diets Containing Proteins of Different Biological Value, J. Nutrition **44**:477, 1951.

82. Sheffner, A. L.; Kirsner, J. B., and Palmer, W. L.: Amino Acid Excretion in Patients with Gastrointestinal Disease During Ingestion of Various Protein Supplements, Gastroenterology **16**:757, 1950.

83. Hempelmann, L. H.; Lisco, H., and Hoffman, J. G.: The Acute Radiation Syndrome: A Study of 9 Cases and a Review of the Problem, Ann. Int. Med. **36**:279, 1952.

84. Wallraff, E. B.; Brodie, E. C., and Borden, A. L.: Urinary Excretion of Amino Acids in Pregnancy, J. Clin. Invest. **29**:1542, 1950.

85. Hofstatter, L.; Ackermann, P. G., and Kountz, W. B.: The Plasma Levels of 9 Free Amino Acids in Old Men and Women, J. Lab. & Clin. Med. **36**:259, 1950.

86. Berlin, N. I.; Tolbert, B. M., and Lawrence, J. H.: Studies in Glycine-2-C^{14} Metabolism in Man: I. The Pulmonary Excretion of $C^{14}O_2$, J. Clin. Invest. **30**:73, 1951.

87. Grossman, C. M., and Hauschildt, J. D.: The Incorporation of C^{14} Glycine into Protein by Human Liver Slices, J. Clin. Invest. **31**:192, 1952.

88. Grinstein, M.; Aldrich, R. A.; Hawkinson, V.; Lowry, P., and Watson, C. J.: Photosensitive or Congenital Porphyria with Hemolytic Anemia: II. Isotopic Studies of Porphyrin and Hemoglobin Metabolism, Blood **6**:699, 1951. Lowry, P. T.; Hawkinson, V., and Watson, C. J.: An Isotopic Study of Type III Porphyrins and Hemoglobin Metabolism in an Unusual Case of "Mixed" Porphyria, Metabolism **1**:149, 1952.

by Chandran and Damodaran.[89] Leucine and histidine tolerance and the rate of renal clearance have been determined by Steele and Le Bovit.[90] Blood cystine levels of patients with cystinuria have not been found to differ significantly from those of normal subjects, supporting the view that cystinuria arises from a peculiarity of renal function rather than from a disturbance of the intermediary metabolism of cystine.[91] Observations on the production and prevention of methionine toxicity in rats have been reported by Harden and Love.[92]

Determinations of the amino acid content of human milk [93] and of certain processed meats [94] have been carried out. Pecora and Hundley [95] have studied the effects of supplementation of polished rice with amino acids.

Kwashiorkor.—Davies [96] has presented an interesting discussion on kwashiorkor, or malignant malnutrition, as seen in African children and has emphasized the occurrence of atrophy of the acinar cells of the pancreas, which may precede the finding of fatty infiltration of the liver and persist long after liver histology has returned to normal. Wills [97] has reviewed the subject of kwashiorkor and reported observations on 36 cases of this syndrome. Hill [98] has reported the results of his studies on liver disease in Jamaican children and has pointed out striking differences from the syndrome of kwashiorkor as seen in other parts of the world. The diet in his group of children was deficient in protein, particularly protein of animal origin, as is the case in kwashiorkor. The clinical picture, however, was usually that of a child who appeared well nourished but had marked enlargement of the liver and ascites. Generalized edema, diarrhea, and changes in the hair and skin, prominent findings in kwashiorkor, were not observed. Hypoalbuminemia and hypochromic anemia were commonly found. Biopsy specimens of the liver seldom revealed any evidence of fatty infiltration, but rather showed evidence of early serous exudation into the perisinusoidal space of Dissé, followed by the appearance of an eosinophilic coagulum in the space and "cuffing" of the central veins, progressing to generalized hepatic fibrosis.

89. Chandran, K., and Damodaran, M.: Amino-Acids and Proteins in Haemoglobin Formation: 2. Isoleucine, Biochem. J. **49**:393, 1951.

90. Steele, B. F., and Le Bovit, C. B.: Leucine and Histidine Tolerance in the Human, J. Nutrition **45**:235, 1951.

91. Fowler, D. I.; Harris, H., and Warren, F. L.: Plasma Cystine Levels in Cystinuria, Lancet **1**:544, 1952.

92. Harden, J. O., and Love, E. L.: Prevention of *dl*-Methionine Toxicity in Rats by Vitamins E, B_{12}, Folacin, Glycine and Arginine, Proc. Exper. Biol. & Med. **78**:728, 1951.

93. Miller, S., and Ruttinger, V.: Essential Amino Acids in Mature Human Milk, Proc. Soc. Exper. Biol. & Med. **77**:96, 1951.

94. Schweigert, B. S.; Bennett, B. A.; McBride, B. H., and Guthneck, B. T.: Amino Acid Content of Processed Meats, J. Am. Dietet. A. **28**:23, 1952.

95. Pecora, L. J., and Hundley, J. M.: Nutritional Improvement of White Polished Rice by the Addition of Lysine and Threonine, J. Nutrition **44**:101, 1951.

96. Davies, J. N. P.: Kwashiorkor, in Conference on Liver Injury, Transactions of the 9th Conference, New York, Josiah Macy, Jr., Foundation, 1950, p. 151.

97. Wills, L.: The Clinical Picture in Children Fed After Weaning on a Predominantly Vegetable Diet, Brit. J. Nutrition **5**:265, 1951.

98. Hill, K. R.: Liver Disease in Jamaican Children, in Conference on Liver Injury, Transactions of the 10th Conference, New York, Josiah Macy, Jr., Foundation, 1951, p. 263.

NUTRITION IN LIVER DISEASE

The importance of nutritional factors in liver disease has been reemphasized by Olsen,[99] who made detailed studies of the dietary histories of a group of 100 patients with cirrhosis and compared them with those of a group of 200 noncirrhotic control subjects. The commonest deficiencies noted in this survey were those of protein and of foodstuffs rich in vitamin B complex. This combination of deficiencies was found in 45% of the group with cirrhosis, but in only 5 to 6% of the controls. Davis[100] has evaluated the results of therapy in cirrhosis as determined by serial liver biopsies and liver-function tests and feels that in most instances the prescription of a diet which provides 150 gm. of protein daily, with modest vitamin supplements, is sufficient. He recommends the use of methionine only in those patients with severe cirrhosis and fatty infiltration of the liver who do not eat well.

Cornatzer and Cayer[101] have studied the effects of choline and methionine upon phospholipid turnover in normal subjects and in patients with hepatitis or cirrhosis. They have been unable to demonstrate any effect of a single large dose of either choline or methionine upon the turnover rate either in normal subjects or in patients with acute hepatitis. When normal subjects were maintained on a low-protein diet for seven days, perhaps too short a period, no effect was observed upon phospholipid turnover after administration of a single 10-gm. dose of choline or methionine. Phospholipid turnover rates in nine cases of untreated cirrhosis were found to be within normal limits, and no change in rate could be demonstrated after eight weeks of treatment with 3 gm. of choline or methionine per day, despite clinical improvement. However, in a group of 20 patients with cirrhosis given a single 10-gm. dose of choline or methionine, a definite increase in rate of phospholipid turnover could be demonstrated in those subjects who had evidence of fatty infiltration of the liver on their initial biopsy. As fat disappeared from the liver after therapy, this effect could no longer be demonstrated. They concluded that choline and methionine have a limited effect in the treatment of patients with fatty livers, and that if the patient is able to eat, the result of dietary therapy alone, over a month, will be the same with or without provision of added choline or methionine. They did feel, however, that in two patients with fatty livers who were admitted in coma the administration of lipotropic agents might have been lifesaving.

99. Olsen, A. Y.: A Study of Dietary Factors, Alcoholic Consumption and Laboratory Findings in 100 Patients with Hepatic Cirrhosis and 200 Non-Cirrhotic Controls, Am. J. M. Sc. **220**:477, 1950.

100. Davis, W. D., Jr.: A Critical Evaluation of Therapy in Cirrhosis of the Liver, South. M. J. **44**:577, 1951.

101. Cornatzer, W. E., and Cayer, D.: The Effects of Lipotropic Factors on Phospholipide Turnover in the Plasma of Normal Persons as Indicated by Radioactive Phosphorus, J. Clin. Invest. **29**:534, 1950; The Effects of Lipotropic Factors on Phospholipide Turnover in the Plasma of Patients with Cirrhosis of Liver, as Indicated by Radioactive Phosphorus, ibid. **29**: 542, 1950. Cayer, D., and Cornatzer, W. E.: The Effects of Lipotropic Factors on the Phospholipide Turnover in Patients with Infectious Hepatitis, Gastroenterology **18**:79, 1951. Cornatzer, W. E.; Cayer, D., and Lambeth, W. A., Jr.: The Effect of a Single Large Dose of Choline or Methionine on Phospholipid Turnover in Normal Human Subjects Maintained on a Low-Protein Diet for 7 Days, J. Lab. & Clin. Med. **38**:705, 1951. Cayer, D.: Action of Lipotropic Factors in Man, in Conference on Liver Injury, Transactions of the 10th Conference, New York, Josiah Macy, Jr., Foundation, 1951, p. 91. Action of Lipotropic Substances in Liver Disease as Measured by Radioactive Phosphorus, editorial, J. A. M. A. **144**:1566, 1950.

The role of vitamin B_{12} as a protective factor against liver injury due to dietary deficiency or exposure to toxic agents and the relation of this vitamin to various lipotropic factors have been the subjects of extensive investigation. Koch-Weser and associates [102] report that rats treated with vitamin B_{12} prior to exposure to carbon tetrachloride show less evidence of liver damage than do untreated control animals. Because of the relatively large doses of the vitamin required to demonstrate this effect, they feel that their results indicate a pharmacologic action rather than a primary vitamin effect. György [103] has demonstrated that vitamin B_{12}, in similarly large doses, shows marked lipotropic activity, either alone or as a supplement to methionine, in rats fed a low-protein, high-fat diet. He was unable to demonstrate any protective action of the vitamin against the hepatic necrosis produced by a yeast diet. Burns and McKibbin [104] have shown that vitamin B_{12} will cure the fatty liver produced in dogs by a choline-deficient diet, and that dietary deficiency of this vitamin in weanling puppies is associated with growth impairment, evidence of liver dysfunction, and, in some instances, gross fatty infiltration of the liver. Strength and associates [105] have demonstrated that the choline requirement for maintenance of normal amounts of liver fat in the rat is greatly reduced by supplementing the diet with vitamin B_{12} and folic acid (folacin). They have further shown that these two vitamins increase the efficiency of betaine, *dl*-methionine, dimethylaminoethanol, and methylaminoethanol as replacements for choline in protecting rats against fatty changes in the liver and renal damage. Hove and Hardin [106] have found that vitamin B_{12} and vitamin E can replace each other in promoting protein utilization and in protecting the liver against carbon tetrachloride toxicity in young rats reared on a low-protein diet deficient in these factors. Folic acid supplements alone had no such effect but were effective in the presence of either vitamin B_{12} or vitamin E. Cartwright and associates [107] have shown that vitamin B_{12} appears to prevent but not reverse the severe liver damage produced in swine by a high-fat diet which presumably contained adequate amounts of protein, vitamin E, choline, and methionine.

György and associates [108] have investigated the effects of various antibiotics upon experimental liver injury produced in rats by "necrogenic" and by high-fat,

102. Koch-Weser, D.; Szanto, P. B.; Farber, E., and Popper, H.: Further Investigation on the Effect of Vitamin B_{12} Concentrate upon Hepatic Injury Produced by Carbon Tetrachloride, J. Lab. & Clin. Med. **36**:694, 1950.

103. György, P.: Vitamin B_{12}, Folic Acid, and the Lipotropic Agents, in Conference on Liver Injury, Transactions of the 9th Conference, New York, Josiah Macy, Jr., Foundation, 1950, p. 207.

104. Burns, M. M., and McKibbin, J. M.: The Lipotropic Effect of Vitamin B_{12} in the Dog, J. Nutrition **44**:487, 1951.

105. Strength, D. R.; Schaefer, E. A., and Salmon, W. D.: The Relation of Vitamin B_{12} and Folacin to the Utilization of Choline and Its Precursors for Lipotropism and Renal Protection in Rats, J. Nutrition **45**:329, 1951.

106. Hove, E. L., and Hardin, J. O.: Effect of Vitamins E, B_{12} and Folacin on CCl_4 Toxicity and Protein Utilization in Rats, Proc. Soc. Exper. Biol. & Med. **77**:502, 1951.

107. Cartwright, G. E.; Tatting, B.; Robinson, J.; Fellows, N. M.; Gunn, F. D., and Wintrobe, M. M.: Hematologic Manifestations of Vitamin B_{12} Deficiency in Swine, Blood **6**:867, 1951.

108. György, P.; Stokes, J., Jr., and Goldblatt, H.: Antimicrobial Agents in the Prevention of Experimental Dietary Injury to the Liver, Tr. A. Am. Physicians **64**:289, 1951. György, P., in discussion at Conference on Liver Injury, New York, May 21-22, 1951, in Transactions of the 10th Conference, New York, Josiah Macy, Jr., Foundation, 1951, p. 133.

low-protein, choline-free diets. They find that streptomycin, terramycin, and, more particularly, aureomycin are definitely effective in preventing necrosis or the development of cirrhosis, ascites, and renal damage in their experimental animals, and in potentiating the effect of methionine as a lipotropic agent. These results suggest that the protective effect is mediated through alterations in the bacterial flora of the bowel. Confirmation of this was obtained in studies with germ-free animals, which survived twice as long as control animals maintained on the same "necrogenic" diet and which showed no evidence of hepatic necrosis at autopsy. The exact role played by intestinal bacteria in the production of experimental dietary liver necrosis or cirrhosis is not known; it is postulated that they may destroy some essential dietary factor, possibly vitamin B_{12} or methionine, but not choline. Sutherland [109] was unable to demonstrate any change in liver fat of dogs or rats on adequate diets when aureomycin was given for as long as 70 days. Two grams of aureomycin taken orally for 30 days by three healthy young men produced no significant change in results of liver-function tests. Sborov [110] and Yesner [111] have indicated that in some patients with hepatic disease or serious infection, fatty liver develops during aureomycin or terramycin therapy despite adequate diets.

Gross and his associates [112] have shown that there is an increased loss of fat in the stools which is not associated with an increase in fecal nitrogen in patients with cirrhosis or hepatitis. No evidence of a deficiency of external pancreatic secretion was demonstrable to account for this, and it was postulated that defective formation and excretion of bile salts by the liver might be responsible. No positive evidence was offered in support of this thesis. The effects of caloric loss from such steatorrhea upon nitrogen balance remain to be evaluated.[113] Farber and Popper [114] and Goldberg and his associates [115] have shown that ethionine, a methionine antagonist, produces acute pancreatic necrosis not involving the islet cells in both male and female rats and fatty infiltration of the liver chiefly in female rats. These changes can be prevented by the administration of methionine, but not by choline. Farber and associates [116] found that fatty changes in the liver could be produced by ethionine in male rats after orchiectomy. Testosterone propionate protected both female and

109. Sutherland, D. A.; Mann, J. D.; Giges, B., and Seligson, D.: Effect of Aureomycin on Liver Fat and Liver Function, Proc. Soc. Exper. Biol. & Med. **77**:458, 1951.

110. Sborov, V. M., in discussion at Conference on Liver Injury, New York, May 21-22, 1951, in Transactions of the 10th Conference, New York, Josiah Macy, Jr., Foundation, 1951, p. 138.

111. Yesner, R., and Kunkel, P.: Preliminary Observations on the Effect of Aureomycin, Terramycin, Tibione, Combined Tibione and Streptomycin and Chloromycetin on the Morphology of the Liver in Man, Yale J. Biol. & Med. **23**:299, 1951.

112. Gross, J. B.; Comfort, M. W.; Wollaeger, E. E., and Power, M. H.: Total Solids, Fat and Nitrogen in the Feces: V. A Study of Patients with Primary Parenchymatous Hepatic Disease, Gastroenterology **16**:140, 1950; Study of Fecal Excretion of Fat and Nitrogen and External Pancreatic Function in Cases of Primary Parenchymatous Hepatic Disease, Proc. Staff Meet., Mayo Clin. **26**:9, 1951.

113. Fecal Nitrogen and Fat in Liver Disease, Nutrition Rev. **9**:187, 1951.

114. Farber, E., and Popper, H.: Production of Acute Pancreatitis with Ethionine and Its Prevention by Methionine, Proc. Soc. Exper. Biol. & Med. **74**:838, 1950.

115. Goldberg, R. C.; Chaikoff, I. L., and Dodge, A. H.: Destruction of Pancreatic Acinar Tissue by *dl*-Ethionine, Proc. Soc. Exper. Biol. & Med. **74**:869, 1950.

116. Farber, E.; Koch-Weser, D., and Popper, H.: The Influence of Sex and of Testosterone upon Fatty Liver Due to Ethionine, Endocrinology **48**:205, 1951.

castrated male rats against the fatty infiltration. Wachstein and Meisel [117] have found that after protein depletion of male rats pancreatic necrosis produced by ethionine is accentuated and fatty infiltration of the liver is observed. The role of the pancreas in the pathogenesis of human hepatic cirrhosis seems in need of further clarification.[118]

Studies by Kirsner [119] suggest that amino acids, with the possible exception of methionine, are metabolized adequately, although less efficiently than normal, in patients with severe liver disease. Concentrations of methionine in plasma during fasting were found to be greatly increased in patients with severe acute hepatitis. Gabuzda and associates [120] were unable to demonstrate any effect of methionine or choline upon nitrogen balance in five patients with cirrhosis and one with acute hepatitis maintained on diets furnishing 0 to 100 gm. of protein daily. They found that increased nitrogen retention followed the administration of testosterone, but that the most marked effects followed increases in dietary protein of 25 to 50 gm. per day. Nitrogen-balance studies in patients with cirrhosis were also conducted by Cook and Van Auken.[121]

Kark [122] has pointed out the rational basis for the use of low-sodium, high-protein diets in the therapy of cirrhosis of the liver complicated by ascites and has reemphasized the fact that protein depletion and sodium retention are only two of many factors which may be operative in the formation of ascitic fluid. Eisenmenger and associates [123] and Lowe and Overy [124] have emphasized the value of low-sodium diets in the control of ascites. Havens and Bluemle [125] reported satisfactory results from administration of human serum albumin and mercurial diuretics in the management of ascites due to cirrhosis. Moser and associates [126] found that ascites and edema were well controlled in eight patients with cirrhosis by the use of an ion-

117. Wachstein, M., and Meisel, E.: Protein Depletion Enhances Pancreatic Damage Caused by Ethionine, Proc. Soc. Exper. Biol. & Med. **77**:569, 1951.

118. Nutrition in Liver Disease, Nutrition Rev. **9**:91, 1951.

119. Kirsner, J. B.; Sheffner, A. L.; Palmer, W. L., and Bergeim, O.: Amino Acids in Plasma and Urine of Patients with Hepatitis Before and After a Single Infusion of Protein Hydrolysate, J. Lab. & Clin. Med. **36**:735, 1950.

120. Gabuzda, G. J., Jr.; Eckhardt, R. D., and Davidson, C. S.: Effect of Choline and Methionine, Testosterone Propionate and Dietary Protein on Nitrogen Balance in Patients with Liver Disease, J. Clin. Invest. **29**:566, 1950.

121. Cook, W. R., and Van Auken, H. A.: Nitrogen Balance Studies in Laennec's Cirrhosis of the Liver, Ann. Int. Med. **34**:1404, 1951.

122. Kark, R. M.; Keeton, R. W.; Calloway, N. O.; Morey, G. R.; Chapman, R. A., and Kyle, R. H.: A Rational Basis for the Use of Low Sodium, High Protein Diet Therapy in Laennec's Cirrhosis, A. M. A. Arch. Int. Med. **88**:61, 1951.

123. Eisenmenger, W. J.; Blondheim, S. H.; Bongiovanni, A. M., and Kundel, H. G.: Electrolyte Studies on Patients with Cirrhosis of the Liver, J. Clin. Invest. **29**:1491, 1950.

124. Lowe, C. R., and Overy, D. C.: An Evaluation of Rigid Dietary Sodium Restriction in Cirrhosis of the Liver, Ann. Int. Med. **31**:1396, 1951.

125. Havens, W. P., Jr., and Bluemle, L. W.: The Effect of Human Serum Albumin and Mercurial Diuretics on Ascites in Patients with Hepatic Cirrhosis, Gastroenterology **16**:455, 1950.

126. Moser, R. H.; Rosenak, B. D.; Pickett, R. D., and Fisch, C.: The Role of Resins in the Treatment of Water Retention Associated with Cirrhosis of the Liver, Gastroenterology **19**:336, 1951.

exchange resin. Transient changes in serum electrolyte concentrations were observed in three patients during therapy. Amatuzio and associates [127] noted that serum concentrations of sodium, potassium, calcium, and phosphorus were lower than normal in 15 patients with decompensated alcoholic cirrhosis prior to the establishment of an adequate diet. Blood pyruvic acid levels were found to be elevated. When an adequate diet was instituted, rapid clinical and chemical recovery ensued. They suggest that the deficiency in calcium and phosphorus may be the result of increased fecal loss of fat. In other studies of alterations in the levels of pyruvic acid in blood and spinal fluid, Amatuzio and Nesbitt [128] found no changes in uncomplicated hepatitis. In hepatic coma, whether the result of cirrhosis or of hepatitis, marked elevations of pyruvic acid were observed in both blood and spinal fluid. Concentrations tended to vary with the clinical state of the patient and were not affected by large doses of thiamine. They suggested that these findings might be explained as the result of failure of the liver to assimilate pyruvic acid to form the dicarboxylic acids necessary for the Krebs cycle. Such a failure in the mechanisms of cellular aerobic metabolism might then explain the neurological and renal manifestations of hepatic coma. Alternatively, it has been suggested that these findings might be produced by a partial deficiency of cocarboxylase.[129]

CARBOHYDRATE NUTRITION

It is beyond the scope of this paper to review contributions made to the knowledge of carbohydrate metabolism and nutrition in diabetes mellitus. It is of interest, however, to note some recent studies on the metabolism of fructose and invert sugar in both normal and diabetic subjects. Weichselbaum and associates [130] have investigated the role of the splanchnic bed in the absorption of fructose administered intravenously to normal subjects. They have also compared the rate of assimilation of glucose with that of fructose in patients with diabetic acidosis and in patients who have been subjected to total pancreatectomy. They found that fructose is assimilated more rapidly than glucose and that its metabolism appears to be independent of the action of insulin. These findings have been confirmed and extended by other investigators,[131] who have demonstrated that fructose given intravenously to diabetic

127. Amatuzio, D. S.; Stutzman, F.; Shrifter, N., and Nesbitt, S.: A Study of Serum Electrolytes (Na, K, Ca, P) in Patients with Severely Decompensated Portal Cirrhosis of the Liver, J. Lab. & Clin. Med. **39**:26, 1952.

128. Amatuzio, D. S., and Nesbitt, S.: A Study of Pyruvic Acid in the Blood and Spinal Fluid of Patients with Liver Disease With and Without Hepatic Coma, J. Clin. Invest. **29**:1486, 1950.

129. Blood Pyruvate Concentration in Liver Coma, Nutrition Rev. **9**:215, 1951.

130. Mendeloff, A. I., and Weichselbaum, T. E.: The Role of the Splanchnic Bed in the Metabolism of Fructose Intravenously Administered to Normal Man, J. Lab. & Clin. Med. **38**:929, 1951. Weichselbaum, T. E., and Daughaday, W. H.: The Rate of Assimilation of Fructose Following the Intravenous Administration to Patients with Diabetic Acidosis and Total Pancreatectomy, ibid. **38**:958, 1951.

131. Craig, J. W.; Drucker, W. R.; Miller, M.; Owens, J. E., and Woodward, H., Jr.: The Metabolism of Fructose in the Liver of Normal and Diabetic Subjects, J. Lab. & Clin. Med. **38**:800, 1951. Miller, M.; Drucker, W. R.; Owens, J. E., and Woodward, H., Jr.: Comparison of the Metabolic Effects of Fructose and Glucose Administered Intravenously to Normal and Diabetic Subjects, ibid. **38**:931, 1951. Miller, M.; Drucker, W. R.; Owens, J. E.; Craig, J. W., and Woodward, H., Jr.: Metabolism of Intravenous Fructose and Glucose in Normal and Diabetic Subjects, J. Clin. Invest. **31**:115, 1952.

subjects produced changes in blood pyruvic acid, citric acid, and inorganic phosphorus comparable with those produced in normal subjects, whereas glucose did not. The rise in blood glucose following the administration of fructose was greater in diabetics than in normal subjects.

Weinstein [132] has investigated the use of solutions of invert sugar in carbohydrate therapy of nondiabetic subjects and feels that such solutions are preferable to dextrose solutions because of more rapid assimilation by the body and smaller loss of sugar in the urine. Lawton and his associates [133] have carried out similar studies and have shown that there is little advantage in using invert sugar in 5% solutions, but that 10% solutions could be given more rapidly, were more completely utilized, and appeared to exert less diuretic effect than corresponding solutions of dextrose. Two thousand cubic centimeters of a 10% solution of invert sugar furnished approximately 100 more calories than did the same amount of dextrose.

Smith [134] has studied concentration of pyruvic acid in arterial blood in aged men following the intravenous injection of dextrose and has concluded that insulin deficiency is not the primary factor involved in the production of prolonged dextrose-tolerance curves observed in senile subjects. Bryant and associates [135] have studied the effects of aging and of various types of breakfasts on the blood sugar levels of normal subjects. They found that the amount of protein in the meal significantly affected the blood sugar level, and that the blood sugar curves of aged subjects did not differ from those of young persons following a similar meal. Brien [136] has compared the results of oral dextrose- and xylose-tolerance tests and concludes that the latter is the more reproducible test of carbohydrate absorption.

VITAMIN A

An excellent discussion on the storage, distribution, and metabolism of vitamin A in health and disease has been presented by Moore and Sharman.[137] Goodwin [138] has discussed the various vitamin A-active substances and reviewed their chemical structure and activity. Leitner [139] has reviewed the principal pathology of vitamin A

132. Weinstein, J. J.: Tolerance of Human Beings to Intravenous Infusions of 15% Invert Sugar, J. Lab. & Clin. Med. **38**:70, 1951; Comparative Utilization of Invert Sugar and Dextrose in Nondiabetic Human Beings, M. Ann. District of Columbia **20**:355, 1951.

133. Lawton, B. R.; Curreri, A. R., and Gale, J. W.: Invert Sugar Solutions for Parenteral Feeding of Surgical Patients, A. M. A. Arch. Surg. **63**:561, 1951.

134. Smith, L. E.: Blood Pyruvate Levels Following Intravenous Glucose Injections in Aged Males, Am. J. M. Sc. **220**:78, 1950.

135. Bryant, K.; Martin, C.; Schumacher, M. T.; Daum, K., and Tuttle, W. W.: Effect of Aging and Various Types of Breakfasts on the Blood Sugar Level, J. Am. Dietet. A. **28**:17, 1952.

136. Brien, F. S.; Turner, D. A.; Watson, E. M., and Geddes, J. H.: A Study of Carbohydrate and Fat Absorption from the Normal and Diseased Intestine in Man: I. The Absorption and Excretion of *d*-Xylose, Gastroenterology **20**:286, 1952.

137. Moore, T., and Sharman, I. M.: Vitamin A Levels in Health and Disease, Brit. J. Nutrition **5**:119, 1951.

138. Goodwin, T. W.: Vitamin A Active Substances, Brit. J. Nutrition **5**:94, 1951.

139. Leitner, Z. A.: Pathology of Vitamin A Deficiency and Its Clinical Significance, Brit. J. Nutrition **5**:130, 1951; The Clinical Significance of Vitamin A Deficiency, Brit. M. J. **1**:1110, 1951.

deficiency, particularly in relation to febrile diseases and diseases of the liver, eyes, and skin. He has pointed out the possible role of maternal vitamin A deficiency in enhancing the frequency of congenital malformations and neonatal death. He suggests that it might prove advantageous to give 40,000 to 50,000 I. U. of vitamin A daily in rheumatic fever and in pregnancy complicated by febrile illnesses, particularly during the first trimester.

Szymanski and Longwell [140] have studied the levels of vitamin A and carotene in plasma in a group of 95 healthy children ranging in age from 2 days to 16 years. Both vitamin A and carotene levels were higher in female than in male infants. Carotene levels in both sexes dropped just before the start of adolescence. Plasma vitamin A levels showed only slight correlation with the amount of vitamin A concentrates given, whereas plasma carotene levels had a much higher degree of correlation with the intake of carotene from plant sources and showed a distinct seasonal variation.

The site of conversion of carotene to vitamin A has been studied by Kon and Thompson,[141] who conclude that β-carotene is transformed in the intestinal wall to vitamin A ester, probably through retinene. Paul and associates [142] have studied the effect of liquid petrolatum (mineral oil) upon β-carotene in vitro and in vivo and have found that isomers of carotene are formed which possess lowered biological activity. Steigmann [143] has found that the effect of liquid petrolatum upon concentrations of vitamin A in plasma depends upon the amount of liquid petrolatum administered and the relation of the time of administration to meals. Plasma vitamin A levels were significantly lowered by liquid petrolatum given in a single daily dose at noontime for four weeks, but not by the same dose given at bedtime.

The role of vitamin A in the formation of visual purple was reviewed and discussed by Morton.[144] Platt [145] has found that the excessive cornification of vaginal epithelium noted in women past the menopause can be modified by the administration of vitamin A and suggests that vitamin A deficiency is of etiological significance in this condition. Hickey [146] has postulated that chalazion may be related to vitamin A deficiency and has reported beneficial effects following administration of vitamin A, 50,000 to 100,000 I.U. daily, for several weeks. No untoward effects were observed with this dosage except for transient diarrhea in a few patients. Bair [147] has reported vitamin A poisoning in a child 28 months of age who received 240,000 I. U. of vitamin A daily for at least three months. An unusual aspect was the

140. Szymanski, B. B., and Longwell, B. B.: Plasma Vitamin A and Carotene Determinations in a Group of Normal Children, J. Nutrition **45**:431, 1951.

141. Kon, S. K., and Thompson, S. Y.: Site of Conversion of Carotene to Vitamin A, Brit. J. Nutrition **5**:114, 1951.

142. Paul, M. F.; Ells, V. R., and Paul, H. E.: The Effect of Mineral Oil on Food Utilization: II. Changes of Beta Carotene in Mineral Oil, Am. J. Digest. Dis. **18**:278, 1951.

143. Steigmann, F.; Popper, H.; Dyniewicz, H., and Goldberg, I. M.: Influence of Mineral Oil Ingestion on the Plasma Vitamin A Level, J. Lab. & Clin. Med. **36**:993, 1950.

144. Morton, R. A.: Vitamins A and Vision, Brit. J. Nutrition **5**:100, 1951.

145. Platt, L. I.: Influence of Vitamin A on Post-Menopausal Cornification of the Vaginal Epithelium, Am. J. Clin. Path. **21**:38, 1951.

146. Hickey, C. S.: The Use of Vitamin A in the Prevention and Treatment of Chalazion, Eye, Ear, Nose & Throat Month. **30**:488, 1951.

147. Bair, G.: Chronic Vitamin A Poisoning: Report of a Case, J. A. M. A. **146**:1573, 1951.

presence of bilateral swellings in the temporal region and marked hyperostosis of the undersurface of the occipital bone. Other cases of vitamin A intoxication have been reported.[148]

THIAMINE

Lossy, Goldsmith, and Sarett [149] studied the urinary excretion of five vitamins of the B complex following the administration of an oral test dose to 115 subjects of varying nutritional status. Mean thiamine excretion after the test dose was significantly decreased in patients in whom peripheral neuropathy or beriberi heart disease were the principal manifestations of vitamin B complex deficiency. Eleven of a group of 22 diabetic subjects had clinical evidence of vitamin B complex deficiency, despite the fact that their diets were apparently adequate in these vitamins. The mean excretion of thiamine and riboflavin by the patients with diabetes was significantly higher than that by normal subjects of good nutritional status; mean N'-methylnicotinamide excretion was significantly lower. No correlation between test dose excretion of these vitamins and the duration, severity, or degree of control of the diabetes, the presence or absence of signs of B complex deficiency, or recent change in weight could be found. These results suggest that there is an abnormality in the utilization of B complex vitamins in patients with diabetes mellitus.

Wertz and associates [150] found that utilization of additional calories supplied by alcohol, in rats, did not seem to require thiamine over and above that required in a basal diet as estimated by changes in thiamine stores in the liver. When the same number of additional calories was supplied by dextrose, significant depletion of thiamine occurred. Yudkin [151] observed that rats fed as little as 9% sucrose or dextrose in their diets were not able to survive thiamine depletion as well as those receiving no carbohydrate. The amount of thiamine in the tissues was unaffected by the presence or absence of carbohydrate in the diet, even though those animals receiving carbohydrate were moribund and the other animals were well. Byerrum and Flokstra [152] demonstrated in rats that as the dietary intake of thiamine increased up to 0.2 mg. per 100 gm. of diet the concentration of cocarboxylase also increased in brain, muscle, and liver. Levels of dietary thiamine greater than this produced no further increase in tissue cocarboxylase, although tissue concentration of thiamine continued to increase, except in the brain, with dietary thiamine increases up to 0.4 mg. per 100 gm. of diet. At least 0.2 mg. of thiamine per 100 gm. of diet was required to maintain maximum tissue cocarboxylase concentrations, although only half this amount was required to maintain normal growth.

148. Sulzberger, M. B., and Lazar, M. P.: Hypervitaminosis A: Report of a Case in an Adult, J. A. M. A. **146**:788, 1951. Rineberg, I. E., and Gross, R. J.: Hypervitaminosis A with Infantile Cortical Hyperostosis, ibid. **146**:1222, 1951.

149. Lossy, F. T.; Goldsmith, G. A., and Sarett, H. P.: A Study of Test Dose Excretion of 5 B Complex Vitamins in Man, J. Nutrition **45**:213, 1951.

150. Wertz, A. W.; Van Horn, P. S., and Lloyd, L. E.: The Effect of the Ingestion of Alcohol on the Storage and Excretion of Thiamine, J. Nutrition **43**:181, 1951.

151. Yudkin, J.: The Vitamin B_1 Sparing Action of Fat and Protein: IV. The Effect of Carbohydrate in Diets Deficient in Vitamin B_1 upon the Survival and Vitamin B_1 Content of the Rat, Biochem. J. **48**:608, 1951.

152. Byerrum, R. U., and Flokstra, J. H.: Cocarboxylase and Thiamine in Tissues of Rats Receiving Different Concentrations of Vitamin B_1 in the Diet, J. Nutrition **43**:17, 1951.

Pratt and associates [153] found that supplementation of the diet of nursing mothers with a multiple-vitamin preparation did not stimulate milk production. Thiamine content of milk and urine was increased, although not consistently for all subjects or in proportion to the amount of supplement given.

RIBOFLAVIN

Pollack and Bookman [154] have studied riboflavin excretion as an index of protein catabolism. They found that nutritionally normal persons will usually retain more than 50% of ingested riboflavin when in nitrogen equilibrium and less than 50% when in negative nitrogen balance. Intensely negative nitrogen balance precipitated by surgical procedures, or by insulin withdrawal in diabetic patients, is associated with excretion of riboflavin in amounts many times greater than that ingested. This increase in riboflavin excretion is presumed to be the result of the breakdown of flavoprotein complexes, which are known to decrease in concentration early in the course of protein starvation. These findings suggest that great care should be taken in interpreting the results of urinary riboflavin excretion tests when assaying the nutritional status of a single subject.

Abnormalities in riboflavin excretion in patients with diabetes mellitus were noted by Lossy and associates [149] and are discussed in the section on thiamine.

Brezezinski and associates [155] have noted the frequent occurrence of evidence of riboflavin deficiency during the last trimester of pregnancy in Israeli women and have found that urinary riboflavin excretion during this period is significantly lower than that of nonpregnant subjects with the same riboflavin intake. Urinary levels of riboflavin increased during lactation but remained lower than those of the control group. Clinical evidence of riboflavin deficiency could be produced in a group of pregnant women maintained on a low-riboflavin intake, but not in a group of nonpregnant control subjects.

NICOTINIC ACID (NIACIN)

As noted in last year's review,[156] it has been demonstrated that tryptophane serves as a precursor of nicotinic acid in man and in many species of animals. Recently, Bonner and Yanofsky [157] have summarized knowledge of the biosynthesis of tryptophane and nicotinic acid in Neurospora and in rats and have outlined the probable steps by which tryptophane is converted to nicotinic acid in the rat.

153. Pratt, J. P.; Hamil, B. M.; Moyer, E. Z.; Kaucher, M.; Roderuck, C.; Coryell, M. N.; Miller, S.; Williams, H. H., and Macy, I. G.: Metabolism of Women During the Reproductive Cycle: XVIII. The Effect of Multi-Vitamin Supplements on the Excretion of B Vitamins in Human Milk, J. Nutrition **44**:141, 1951.

154. Pollack, H., and Bookman, J. J.: Riboflavin Excretion as a Function of Protein Metabolism in the Normal, Catabolic and Diabetic Human Being, J. Lab. & Clin. Med. **38**:561, 1951.

155. Brezezinski, A.; Bromberg, Y. M., and Braun, K.: Riboflavin Excretion During Pregnancy and Early Lactation, J. Lab. & Clin. Med. **39**:84, 1952.

156. Goldsmith, G. A., and Gibbens, J.: Recent Advances in Nutrition: Review of the Literature, 1949-1950, A. M. A. Arch. Int. Med. **88**:93, 1951.

157. Bonner, D. M., and Yanofsky, C.: The Biosyntheses of Tryptophan and Niacin and Their Relationships, J. Nutrition **44**:603, 1951.

Pathways for the bacterial oxidation of tryptophane have been surveyed by Stanier and associates.[158]

The role of corn diets in the production of pellagra may be explained in part by the low tryptophane content of corn. Recent findings of Kodicek [159] may shed light on this problem. Maize, in common with other cereals, was found to contain nicotinic acid in bound form which was unavailable to the rat unless previously hydrolyzed by alkali. Maize which had been treated by alkaline digestion became pellagra-preventive for rats, probably because of liberation of free nicotinic acid. Laguna and Carpenter [160] found that the growth depression induced in rats fed high-corn diets could be diminished by steeping corn in limewater, then neutralizing and drying it, a process similar to that used in making *tortillas* in Mexico. It would be interesting to compare the incidence of pellagra in areas where *tortillas* form an important part of the diet with the incidence in areas where maize is consumed in other forms.

The human requirement for nicotinic acid is unknown, owing in large part to the tryptophane-nicotinic acid relationships noted above. Goldsmith and associates [161] produced pellagra in three to four months in two subjects maintained on corn diets which furnished approximately 200 mg. of tryptophane and 5 mg. of nicotinic acid daily. Thus, the minimal nicotinic acid requirement with this diet is greater than 5 mg.

Bean and co-workers [162] noted rapid improvement of pellagrous glossitis following administration of nine hourly doses of 100 mg. of tryptophane in two subjects who were unimproved while receiving a control diet with little nicotinic acid restriction.

In a study of metabolism of B vitamins in newborn infants, Dean and Holman [163] found that the amount of nicotinic acid excreted greatly exceeded the amount ingested during the first week of life. One factor which may partially account for these findings is conversion of the tryptophane in milk to nicotinic acid.

Circulatory effects of intravenous administration of nicotinic acid were investigated by Lequime and associates.[164] Injection of 75 mg. produced vasodilatation of the skin, definite hypotension, acceleration of cardiac rate, and an increase in cardiac output which averaged 71%. The last effect, in particular, indicates that therapy of coronary syndromes with nicotinic acid is undesirable.

Relationships among pyridoxine, nicotinic acid, and tryptophane in metabolism are discussed below.

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158. Stanier, R. Y.; Hayaishi, O., and Tsuchida, M.: The Bacterial Oxidation of Tryptophan: I. A General Survey of the Pathways, J. Bact. **62**:355, 1951.

159. Kodicek, E.: The Biological Activity for the Rat of the Bound Form of Nicotinic Acid Present in Maize, Biochem. J. **48**:viii, 1951.

160. Laguna, J., and Carpenter, K. J.: Raw Versus Processed Corn in Niacin-Deficient Diets, J. Nutrition **45**:21, 1951.

161. Goldsmith, G. A.; Sarett, H. P.; Register, U. D., and Gibbens, J.: Niacin Depletion and Requirement in Man, Federation Proc. **10**:383, 1951.

162. Bean, W. B.; Franklin, M., and Daum, K.: A Note on Tryptophane and Pellagrous Glossitis, J. Lab. & Clin. Med. **38**:167, 1951.

163. Dean, R. F. A., and Holman, W. I. M.: Excretion and Intake of B Vitamins in Newborn Infants, Arch. Dis. Childhood **25**:292, 1950.

164. Lequime, J., and others, cited in Circulatory Effects of Intravenous Injections of Niacin, Nutrition Rev. **9**:138, 1951.

PYRIDOXINE

Studies of pyridoxine balance in human subjects have shown that, except at relatively high levels of intake, less of the vitamin is excreted in known forms than is ingested.[165] Seventy-five to 90% of ingested pyridoxine is excreted in the urine as 4-pyridoxic acid. Fecal excretion of the vitamin was found to remain relatively constant and was not influenced by the amount of pyridoxine ingested or by the administration of succinylsulfathiazole (sulfasuxidine®).

Interrelationships between pyridoxine and nicotinic acid metabolism have been investigated by Heimberg and associates.[166] Administration of tryptophane to pyridoxine-deficient rats leads to an increased urinary excretion of xanthurenic acid, which is diminished by the addition of nicotinic acid to the diet. Pyridoxine did not appear to be directly involved in the conversion of tryptophane to N^1-methylnicotinamide. Williams and associates [167] have observed that pyridoxine deficiency does not disturb the conversion of tryptophane to liver pyridine nucleotides. Nicotinic acid fed to pyridoxine-deficient rats was found to spare liver pyridine nucleotide concentrations.

Carter and Phizackerley [168] found that pyridoxine deficiency in the rat is accompanied with a decrease in total body lipid and in the concentration of neutral fat in the liver. Lipid concentrations could be restored to normal levels by administration of a high-fat diet. Since no impairment of absorption of fat, carbohydrate, or protein could be demonstrated in pyridoxine-deficient animals, these findings suggest the need for further studies of the relation of pyridoxine to the process of fat synthesis in the animal body.

Linkswiler, Baumann, and Snell [169] have observed that rats fed pyridoxal or pyridoxamine grew less than rats fed equivalent amounts of pyridoxine. The relative growth-promoting activities of these three substances were not influenced by the addition of vitamin B_{12} to the basal ration. Aureomycin caused a marked increase in the growth of rats fed limiting amounts of pyridoxal or pyridoxamine. In the presence of the antibiotic, all three forms of vitamin B_6 were of approximately equal growth-promoting activity. It was postulated that aureomycin may prevent utilization or destruction of these growth-promoting factors by intestinal bacteria, and that a similar action directed toward other food factors may explain the growth-promoting effects of antibiotics in crude rations.

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165. Linkswiler, H., and Reynolds, M. S.: Urinary and Fecal Elimination of B_6 and 4-Pyridoxic Acid on 3 Levels of Intake, J. Nutrition **41**:523, 1950; Balance Studies on Vitamin B_6 inMan, Nutrition Rev. **9**:197, 1951.

166. Heimberg, M.; Rosen, F.; Leder, I. G., and Perlzweig, W. A.: The Significance of Dietary Pyridoxine, Niacin and Protein in the Conversion of Tryptophan to N^1-Methylnicotinamide, Arch. Biochem. **28**:225, 1950.

167. Williams, J. N., Jr.; Feigelson, P.; Shahinian, S. S., and Elvehjem, C. A.: Interrelationships of Vitamin B_6, Niacin and Tryptophan in Pyridine Nucleotide Formation, Proc. Soc. Exper. Biol. & Med. **76**:441, 1951.

168. Carter, C. W., and Phizackerley, P. J. R.: The Influence of Pyridoxine on Fat Metabolism in the Rat, Biochem. J. **49**:222, 1951.

169. Linkswiler, H.; Baumann, C. A., and Snell, E. E.: Effect of Aureomycin on the Response of Rats to Various Forms of Vitamin B_6, J. Nutrition **43**:565, 1951.

Ershoff [170] has found that rats raised to maturity on a pyridoxine-deficient diet show decreased resistance to prolonged exposure to cold. Poppen and his associates [171] have described the blood picture of pyridoxine deficiency in the monkey.

PANTOTHENIC ACID

Pantothenic acid has been found to constitute part of the molecule of coenzyme A, which has been shown to play a critical role in intermediate metabolism.[172] Pantothenic acid deficiency has not been observed in man, and the place of this vitamin in clinical medicine has not been determined.

The use of pantothenic acid in the therapy of paralytic ileus has been suggested by the similarity of bowel changes in this condition to those seen in pantothenic acid deficiency in rats. Jacques [173] has reported good results from the intramuscular administration of 50 mg. of calcium pantothenate in 16 cases of paralytic ileus. Hyperesthesia and burning sensations in the lower extremities of patients given fluids parenterally for long periods are reported to respond to the administration of calcium pantothenate after unsuccessful use of a preparation containing other B complex vitamins.[174] Schmidt [175] has been unable to detect any difference in pantothenic acid excretion following an intramuscularly given test dose in patients with gray hair and baldness as compared with a group of normal controls.

FOLIC ACID (FOLACIN, PTEROYLGLUTAMIC ACID) AND CITROVORUM FACTOR (FOLINIC ACID)

The citrovorum factor, also called folinic acid, is a relatively new member of the folic acid group of vitamins. Leucovorin, a synthetic compound with citrovorum factor activity, is thought to have the formula 5-formyl 5, 6, 7, 8-tetrahydropteroylglutamic acid.[176] Natural citrovorum factor which has been isolated from liver appears to have approximately twice the activity by weight of synthetic leucovorin.[177] Citrovorum factor is believed to be a metabolically active form of folic acid. It can replace folic acid in the diet of chicks [178] and overcome the folic acid deficiency induced by "aminopterin" (4-aminopteroylglutamic acid) or sulfonamide drugs in

170. Ershoff, B. H.: Decreased Resistance of Pyridoxine-Deficient Rats to Cold Exposure, Proc. Soc. Exper. Biol. & Med. **78**:385, 1951.

171. Poppen, K. J.; Greenberg, L. D., and Rinehart, J. F.: The Blood Picture of Pyridoxine Deficiency in the Monkey, Blood **7**:436, 1952.

172. Snell, E. E., and Wright, L. D.: The Water-Soluble Vitamins, Ann. Rev. Biochem. **19**:288, 1950.

173. Jacques, J. E.: Pantothenic Acid in Paralytic Ileus, Lancet **2**:861, 1951.

174. Fraser, R., and Flink, E. B.: Magnesium, Potassium, Phosphorus, Chloride, and Vitamin Deficiency as a Result of Prolonged Use of Parenteral Fluids, J. Lab. & Clin. Med. **38**:809, 1951.

175. Schmidt, V.: The Excretion of Pantothenic Acid in Patients with Achromotrichia and Alopecia, J. Gerontol. **6**:369, 1951.

176. Cosulich, D. B.; Roth, B.; Smith, J. M., Jr.; Hultquist, M. E., and Parker, R. P.: Acid Transformation Products of Leucovorin, J. Am. Chem. Soc. **73**:5006, 1951. Flynn, E. H.; Bond, T. J.; Bardos, T. J., and Shive, W.: A Synthetic Compound with Folic Acid Activity, ibid. **73**:1979, 1951.

177. Keresztesy, J. C., and Silverman, M.: Crystalline Citrovorum Factor from Liver, J. Am. Chem. Soc. **73**:5510, 1951. Silverman, M., and Keresztesy, J. C.: Comparison of Citrovorum Factor and a Synthetic Compound with Leuconostoc Citrovorum Growth Activity, ibid. **75**:1897, 1951.

178. Hill, E. G., and Briggs, G. M.: Replacement of Pteroylglutamic Acid by Folinic Acid for the Chick, Proc. Soc. Exper. Biol. & Med. **76**:417, 1951.

rats.[179] Folic acid-citrovorum factor appears to participate in the synthesis of compounds utilized in the formation of nucleoproteins and in transmethylation processes.[180] Shive [181] has recently reviewed the relation of this vitamin to metabolic processes. Welch [182] postulated that citrovorum factor may serve as the prosthetic group of an enzyme or as the precursor of such a group. The labile methyl group can be synthesized by rats (in vivo) and by liver slices (in vitro), the rate of synthesis being influenced by catalysts derived from synthetic folic acid by liver tissues. The addition of "aminopterin" to liver slices prevents conversion of folic acid to citrovorum factor. The toxic effects of "aminopterin" in rats can be completely prevented by administration of citrovorum factor. These findings suggest that the activity of folic acid is dependent upon its conversion to citrovorum factor and that "aminopterin" acts by interfering with this conversion." Williams [183] and Swendseid and associates [184] have pointed out the probable participation of citrovorum factor in several enzyme systems. Bound forms of citrovorum factor are present in natural materials and may be released by enzymatic digestion.[185]

Register and Sarett [186] found that the average urinary excretion of folic acid and citrovorum factor by 14 human subjects on normal diets was 4.1 and 2.6 γ daily, respectively; excretion was increased on high-purine diets. Broquist and associates [187] reported that approximately 0.1% of an oral dose of 50 mg. of folic acid was excreted in the urine as citrovorum factor in the subsequent six hours. Welch and co-workers [188] found that in both rats and human subjects administration of

179. Waisman, H. A.; Cravioto, J.; Green, M.; Remenchik, A., and Richmond, J. B.: Aureomycin and Citrovorum Factor in Sulfa and Aminopterin-Induced Folic Acid Deficiencies, Federation Proc. **10**:266, 1951. Waisman, H. A.; Green, M.; Munoz, J. C.; Remenchik, A., and Richmond, J. B.: Role of Aureomycin and Citrovorum Factor in Folic Acid Deficiencies, Proc. Soc. Exper. Biol. & Med. **76**:384, 1951.

180. Kelley, B.: Effect of in Vitro Citrovorum Factor and Aminopterin on Metabolism of Glycine by Chick Liver Homogenates, Federation Proc. **10**:206, 1951. Lascelles, J.; Cross, M. J., and Woods, D. D.: Leuconostoc Citrovorum Factor and the Synthesis of Serine by Micro-Organisms, Biochem. J. **49**:1xvi, 1951. Williams, J. N., Jr.: Effect of Aminopterin on Choline Betaine Aldehyde and Betaine Metabolism, Proc. Soc. Exper. Biol. & Med. **76**:206, 1951.

181. Shive, W.: The Functions of B-Vitamins in the Biosynthesis of Purines and Pyrimidines, in Vitamins and Hormones: Advances in Research and Applications (1950), edited by R. S. Harris and K. V. Thimann, New York, Academic Press, Vol. 9, 1951, p. 75.

182. Welch, A. D.: New Developments in the Study of Folic Acid, Tr. A. Am. Physicians **63**:147, 1950.

183. Williams, J. N., Jr.: Relation of Leuconostoc Citrovorum Factor to Activity of Liver Choline Oxidase, J. Biol. Chem. **191**:123, 1951.

184. Swendseid, M. E.; Bethell, F. H., and Ackermann, W. W.: The Intracellular Distribution of Vitamin B_{12} and Folinic Acid in Mouse Liver, J. Biol. Chem. **190**:791, 1951.

185. Hill, C. H., and Scott, M. L.: Enzymatic Release of Citrovorum Factor, Federation Proc. **10**:197, 1951. Scheid, H. E., and Schweigert, B. S.: Some Factors Affecting the Potencies of Vitamin B_{12} and Leuconostoc Citrovorum Factor of Certain Natural Products, J. Biol. Chem. **185**:1, 1950.

186. Register, U. D., and Sarett, H. P.: Urinary Excretion of Vitamin B_{12}, Folic Acid, and Citrovorum Factor in Human Subjects on Various Diets, Proc. Soc. Exper. Biol. & Med. **77**:837, 1951.

187. Broquist, H. P.; Stokstad, E. L. R., and Jukes, T. H.: Biochemical Studies with the "Citrovorum Factor," J. Lab. & Clin. Med. **38**:95, 1951.

188. Welch, A. D.; Nichol, C. A.; Anker, R. M., and Boehne, J. W., III: The Effect of Ascorbic Acid on the Urinary Excretion of Citrovorum Factor Derived from Folic Acid, J. Pharmacol. & Exper. Therap. **103**:403, 1951.

ascorbic acid with folic acid increased the excretion of citrovorum factor threefold as compared with administration of folic acid alone. Excretion of citrovorum factor after a dose of leucovorin was not increased by simultaneous administration of ascorbic acid. Broquist and associates [187] confirmed these findings in man. They also showed that when liver homogenates from rats deficient in folic acid were incubated in the presence of folic acid there was a marked increase in the amount of citrovorum factor formed when ascorbic acid was added to the system. This confirmed findings reported earlier by Nichol and Welch.[189]

Leucovorin failed to alleviate dietary ascorbic acid deficiency in guinea pigs,[187] nor did folic acid have an antiscorbutic effect.[190] In human scurvy only small amounts of citrovorum factor were detected in the urine,[191] and excretion increased slowly after oral administration of folic acid. Addition of large doses of ascorbic acid to the regimen led to only a slight increase in citrovorum factor excretion,[188] in contrast to findings in normal subjects. Folic acid corrects the decrease in pentose nucleic acid in liver cells in "megaloblastic monkeys" but not the similar decrease which occurs in starved or scorbutic animals.[192] It is obvious that ascorbic acid is involved in folic acid metabolism, and it appears to have a role in the conversion of folic acid to citrovorum factor. Complete understanding of the mechanisms involved must await further study.

Administration of desiccated thyroid to rats caused an increase in citrovorum factor excretion in normal but not in folic acid-deficient animals.[193] In immature rats fed diets containing folic acid and desiccated thyroid, administration of vitamin B_{12} or whole liver powder led to an increase in citrovorum-factor excretion. Dietrich and associates [194] presented data which indicated that vitamin B_{12} may have a role in the conversion of folic acid to citrovorum factor. The relations between folic acid and vitamin B_{12} are complex and as yet poorly understood. Both factors are important in hematopoiesis, probably in nucleoprotein synthesis, and in the metabolism of labile methyl groups and choline.

Effects of folic acid, ascorbic acid, vitamin B_{12}, and related factors on experimentally induced macrocytic anemia in swine have been studied by Cartwright and associates.[195] In experimental megaloblastic anemia in monkeys, May and

189. Nichol, C. A., and Welch, A. D.: Synthesis of Citrovorum Factor from Folic Acid by Liver Slices: Augmentation by Ascorbic Acid, Proc. Soc. Exper. Biol. & Med. **74**:52, 1950.

190. Silverman, F. N., and Mackler, B.: Lack of Effect of Folic Acid on Scurvy in Guinea Pigs, Proc. Soc. Exper. Biol. & Med. **76**:574, 1951.

191. Welch and others.[188] Gabuzda, G. J., Jr.; Phillips, G. B.; Schilling, R. F., and Davidson, C. F.: Metabolism of Pteroylglutamic Acid and Citrovorum Factor in Human Scurvy, J. Clin. Invest. **30**:639, 1951.

192. Lowe, C. U.; Barnum, C. P., and Terry, W. S.: Certain Quantitative Aspects of Nucleic Acid Metabolism in the Livers of Megaloblastic Monkeys, J. Lab. & Clin. Med. **36**:957, 1950.

193. Drysdale, G. R.; Betheil, J. J.; Lardy, H. A., and Baumann, C. A.: The Excretion of the Citrovorum Factor by Hyperthyroid Rats, Arch. Biochem. **33**:1, 1951.

194. Dietrich, L. S.; Monson, W. J., and Elvehjem, C. A.: Observations on a Relationship Between Vitamin B_{12}, Folic Acid and the Citrovorum Factor, Proc. Soc. Exper. Biol. & Med. **77**:93, 1951.

195. Cartwright, G. E.; Palmer, J. G.; Tatting, B.; Ashenbrucker, H., and Wintrobe, M. M.: Experimental Production of Nutritional Macrocytic Anemia in Swine: III. Further Studies on Pteroylglutamic Acid Deficiency, J. Lab. & Clin. Med. **36**:675, 1950.

co-workers [196] found that small doses of crude folinic acid (citrovorum factor) were at least as effective as folic acid in therapy and that ascorbic acid was not essential for the effect.

Citrovorum factor has been administered orally or parenterally to patients with pernicious anemia in relapse.[197] Hematologic and clinical improvement was observed in nearly all instances and complete remission in some cases. Jarrold and associates [197f] concluded that this factor was no more effective than folic acid given in similar amounts. Meyer [198] administered 6 mg. of citrovorum factor daily for three months to one patient, and, although hematologic response was satisfactory, subacute combined degeneration of the spinal cord developed. In contrast to this, Moore [199] gave a patient with pernicious anemia with severe neurological abnormalities 10 mg. of citrovorum factor daily by mouth and noted marked neurological as well as hematologic improvement. According to Davidson [197d,e] and Spies,[197g] patients with nutritional macrocytic anemia, tropical sprue, and macrocytic anemia of pregnancy have also responded to citrovorum factor. In megaloblastic anemia of infancy citrovorum factor was effective in two cases in amounts of 75 γ daily parenterally; folic acid was effective in two cases in amounts of 200 and 500 γ daily orally.[200] No failure has been reported in the treatment of megaloblastic anemia of infancy with folic acid,[201] although failures have been reported with vitamin B_{12}. Another condition in which folic acid administration appears to be the treatment of choice is macrocytic anemia of pregnancy.[202] The usual therapeutic dose of folic acid is 5 to 15 mg. per day, given either orally or parenterally.

196. May, C. D.; Sundberg, R. D., and Schaar, F.: Comparison of Effects of Folic Acid and Folinic Acid in Experimental Megaloblastic Anemia, J. Lab. & Clin. Med. **36**:963, 1950. May, C. D.; Sundberg, R. D.; Schaar, F.; Lowe, C. U., and Salmon, R. J.: Experimental Nutritional Megaloblastic Anemia: Relation of Ascorbic Acid and Pteroylglutamic Acid; I. Nutritional Data and Manifestations of Animals, A. M. A. Am. J. Dis. Child. **82**:282, 1951.

197. (*a*) Ellison, R. R.; Wolfe, S.; Lichtman, H.; Ginsberg, V., and Watson, J.: Effect of Citrovorum Factor in Pernicious Anemia, Proc. Soc. Exper. Biol. & Med. **76**:366, 1951. (*b*) Meyer, L. M.; Brahin, C. M., and Sawitsky, A.: Treatment of Pernicious Anemia with Citrovorum Factor, ibid. **76**:86, 1951. (*c*) Meyer, L. M.; Diefenbach, W. C. L., and Brahin, C. M.: Intravenous Treatment of Pernicious Anemia with Citrovorum Factor (Leucovorum), ibid. **78**:119, 1951. (*d*) Davidson, L. S. P., and Girdwood, R. H.: Treatment of Pernicious Anemia with Leuconostoc Citrovorum Factor, Lancet **1**:722, 1951; (*e*) Treatment of Megaloblastic Anemias with Citrovorum Factor, ibid. **2**:1193, 1951. (*f*) Jarrold, T.; Horrigan, D.; Thompson, C., and Vilter, R. W.: Folinic Acid and Pernicious Anemia, Science **113**:688, 1951. (*g*) Spies, T. D.; Lopez, G. G.; Milanes, F.; Toca, R. L.; Reboredo, A., and Stone, R. E.: The Response of Patients with Pernicious Anemia with Nutritional Macrocytic Anemia, and with Tropical Sprue to Folinic Acid or Citrovorum Factor, South. M. J. **43**:1076, 1951.

198. Meyer, L. M., and Diefenbach, W. C. L.: Pernicious Anemia Treated with Citrovorum Factor (Leucovorin), Am. J. Clin. Path. **21**:1054, 1951.

199. Moore, M. S.; Scott, M. L.; Wallace, C. S., and Fiala, G.: Orally Administered Citrovorum Factor in the Treatment of Pernicious Anemia with Neurologic Complications, New York J. Med. **51**:2645, 1951.

200. Woodruff, C. W.; Peterson, J. C., and Darby, W. J.: Citrovorum Factor and Folic Acid in Treatment of Megaloblastic Anemia in Infancy, Proc. Soc. Exper. Biol. & Med. **77**:16, 1951.

201. Woodruff, C. W., and Peterson, J. C.: The Treatment of Megaloblastic Anemia in Infancy, Postgrad. Med. **10**:189, 1951.

202. Ginsberg, V.; Watson, J., and Lichtman, H.: Megaloblastic Anemia of Pregnancy: Response to Pteroylglutamic Acid After Failure of Response to Liver Extract and Vitamin B_{12}, J. Lab. & Clin. Med. **36**:238, 1950.

In the maintenance therapy of pernicious anemia, Chodos and Ross [203] found no improvement in hematologic status when treatment with vitamin B_{12} or liver extract was supplemented with folic acid. However, they suggested that therapy with folic acid be supplemented with vitamin B_{12} or liver extract in patients with sprue, nutritional macrocytic anemia, or other macrocytic anemias associated with gastrointestinal pathology, to insure against development of disease of the nervous system. A patient with Gaucher's disease who had macrocytic anemia and megaloblastic bone marrow failed to improve with liver extract but showed a good hematopoietic response to folic acid.[204]

Megaloblastic anemia may be associated with intestinal stricture or anastomosis. In one such case reported by Allen and Critchley [205] relief of intestinal stricture by surgical removal of the lesion led to cure of the anemia without recourse to liver, vitamin B_{12}, or folic acid therapy. Watson and Witts [206] produced experimental macrocytic anemia in rats by formation of a small intestinal cul-de-sac, provided that the cul-de-sac had stagnant contents and was placed in the upper part of the small intestine. The anemia was associated with hemolysis and sometimes with steatorrhea and responded to folic acid or aureomycin, but poorly or not at all to vitamin B_{12}.

Folic acid antagonists have been investigated in the treatment of leukemia, but severe toxicity has limited their usefulness.[207] Recent studies indicate that the toxic effects of "aminopterin" and "a-methopterin" (4-amino-10-N-methylpteroylglutamic acid) can be suppressed or reversed by citrovorum factor.[208] Swendseid and associates [209] have studied folic acid metabolism in leukemia and have suggested metabolic displacement of folic acid by "aminopterin" with perhaps the formation of a stable "aminopterin" complex. Toxicity may be dependent upon the degree of saturation of the tissues with folic acid prior to "aminopterin" therapy.

Spray and co-workers [210] found that some patients with pernicious anemia or steatorrhea excreted less folic acid after intravenous administration of small doses than did normal subjects, suggesting an increase in the utilization of this vitamin.

203. Chodos, R. B., and Ross, J. F.: The Effects of Combined Folic Acid and Liver Extract Therapy, Blood **6**:1213, 1951.

204. Krim, M.; Sawitsky, A.; Krohn, D., and Meyer, L. M.: Gaucher's Disease with Megaloblastic Bone Marrow: Response to Therapy, A. M. A. Arch. Int. Med. **87**:418, 1951.

205. Allen, G., and Critchley, C. F.: Macrocytic Anaemia, Brit. M. J. **1**:67, 1951.

206. Watson, G. M., and Witts, L. J.: Intestinal Macrocytic Anaemia, Brit. M. J. **1**:13, 1952.

207. Folacin Antagonists in the Treatment of Acute Leukemia, Nutrition Rev. **9**:143, 1951.

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209. Swendseid, M. E.; Bethell, F. H., and Bird, O. D.: The Concentration of Folic Acid in Leukocytes: Observations on Normal Subjects and Persons with Leukemia, Cancer Res. **11**:864, 1951. Swendseid, M. E.; Swanson, A. L.; Miller, S., and Bethell, F. H.: The Metabolic Displacement of Folic Acid by Aminopterin: Studies in Leukemia Patients, Blood **7**:302, 1952. Swendseid, M. E.; Swanson, A. L.; Meyers, M. C., and Bethell, F. H.: The Nutritional Status of Folic Acid in Persons with Leukemia and Its Possible Relation to Effects of Aminopterin Therapy, ibid. **7**:307, 1952.

210. Spray, G. H.; Fourman, P., and Witts, L. J.: The Excretion of Small Doses of Folic Acid, Brit. M. J. **2**:202, 1951.

Scudamore and associates [211] noted no significant effect of folic acid given intravenously on cholinesterase activity of blood in normal subjects or in patients with pernicious anemia. The rise in cholinesterase activity which occurs during treatment of pernicious anemia appears to be brought about indirectly by increased erythropoiesis.

VITAMIN B_{12}

The large number of publications dealing with vitamin B_{12} necessitates limiting discussion to the most pertinent contributions. E. Lester Smith [212] reviewed current knowledge of vitamin B_{12} in regard to chemistry, methods of assay, sources, requirements, and mode of action. In common with certain other vitamins, vitamin B_{12} comprises a group of closely related chemical compounds with similar physiologic activities. Vitamins B_{12}, B_{12a}, B_{12b}, B_{12c}, and B_{12d} have been described.[213] Vitamin B_{12} contains the cyan group and has been termed cyanocobalamin; vitamins B_{12a} and B_{12b}, which were shown to be identical, contain the hydroxy group and are termed hydroxocobalamin [214]; vitamin B_{12c} contains the nitrite group.[212] Recent investigation indicates that vitamin B_{12d} is identical with B_{12b}.[215] Assay methods for vitamin B_{12} include microbiological procedures using various strains of lactobacilli, a mutant of Escherichia coli, or Euglena gracilis, the last being the most sensitive,[212] and a chemical method [216] which is relatively insensitive. Various forms of vitamin B_{12} and desoxyribosides can be separated by paper chromatography.[212] Vitamin B_{12b}, vitamin B_{12c}, and a thiocyanate analogue of vitamin B_{12} have an effect equal to that of vitamin B_{12} in the treatment of pernicious anemia.[217] Hausmann [218] obtained a hematopoietic response and change of the megaloblastic bone marrow to normal in two patients with pernicious anemia following administration of 2.0 and 2.8 gm. of thymidine, respectively. These findings were interpreted as supporting the assumption that vitamin B_{12} exerts an enzyme influence on nuclear metabolism.

211. Scudamore, H. H.; Gabuzda, G. J., and Vorhaus, L. J.: Effect of Intravenous Folic Acid on the Cholinesterase Activity of the Blood and the Folic Acid Blood Levels, J. Lab. & Clin. Med. **38**:183, 1951.

212. Smith, E. L.: Vitamin B_{12}: Part I, Nutrition Abst. & Rev. **20**:795, 1950-1951.

213. Smith, E. L.: The Vitamin B_{12} Group of Factors, Brit. M. J. **1**:151, 1951.

214. Kaczka, E. A.; Wolf, D. E.; Kuehl, F. A., Jr., and Folkers, K.: Vitamin B_{12}: Reactions of Cyano-Cobalamin and Related Compounds, Science **112**:354, 1950.

215. Smith, E. L.: Vitamin B_{12d}, Chemistry & Industry, 1951, Vol. 70, p. 939; Pitfalls in Partition Chromatography, Nature, London **169**:60, 1952.

216. Boxer, G. E., and Rickards, J. C.: Chemical Determination of Vitamin B_{12}: I. Determination of 5, 6-Dimethyl-Benzimidazole by Colorimetric and Fluorimetric Methods, Arch. Biochem. **29**:75, 1950.

217. Ungley, C. C.: Effect of Vitamin B_{12c} in Pernicious Anaemia and Subacute Combined Degeneration of the Cord, Brit. M. J. **1**:152, 1951. Chalmers, J. N. M.: Haemopoietic Activity of Vitamins B_{12c} and B_{12d} in Pernicious Anaemia, ibid. **1**:161, 1951. Schilling, R. F.; Harris, J. W., and Castle, W. B.: Observations on the Etiologic Relationship of Achylia Gastrica to Pernicious Anemia: XIII. Hematopoietic Activity of Vitamin B_{12a} (Vitamin B_{12b}), Blood **6**:228, 1951. James, G. W., III, and Abbott, L. D., Jr.: Effectiveness of Thiocyanate Analog of Vitamin B_{12} in Pernicious Anemia, Proc. Soc. Exper. Biol. & Med. **77**:416, 1951.

218. Hausmann, K.: Haemopoietic Effect of Thymidine in Pernicious Anaemia, Lancet **1**:329, 1951.

Vitamin B_{12} has been found in nature in complex or "bound" forms which may be inactive either clinically or microbiologically. Hausmann and Mulli [219] isolated cobalt-containing pigments from fish solubles which had high microbiological activity but were without hematopoietic effect in pernicious anemia unless preliminary treatment with potassium cyanide had been carried out. Pennington [220] postulated a heat-labile vitamin B_{12} complex in rat feces in view of the marked increase in microbiological activity of fecal extracts following heating. A heat-labile microbiologically inactive complex of vitamin B_{12} is formed with a factor in gastric juice, and a similar complex may be present in blood sera. A number of other substances have been shown to combine with vitamin B_{12}, affecting biological activity.[212]

The best sources of vitamin B_{12} are liver extract, Streptomyces griseus, and press juice from autoclaved fish.[221] Of foodstuffs, fish meal, liver, and related products contain appreciable amounts of vitamin B_{12}, while cereals contain little or none.[222] Milk from various species contains 0.12 to 6.6 γ of vitamin B_{12} per liter.[223]

Of the several functions of vitamin B_{12}, the hematopoietic effects have been most widely studied and are of the greatest interest in human nutrition at present. Ungley [224] has written an admirable review of the clinical aspects of vitamin B_{12} which should be read by anyone undertaking clinical research in this field. Several excellent summaries of present concepts of the etiology and pathogenesis of megaloblastic anemias have been published.[225] There is abundant evidence that vitamin B_{12} is the anti-pernicious-anemia factor of liver and also the "extrinsic" factor of food. The nature and exact role of the "intrinsic" factor of gastric juice have not been established. Intrinsic factor appears to facilitate the absorption of vitamin B_{12} from the intestinal tract, although the mechanism involved remains conjectural. Details concerning relationships between intrinsic factor and vitamin B_{12} are found in publications of Ungley,[224] Hall,[226] and Bethell.[227] Gastric juice contains a substance which binds vitamin B_{12}, rendering it microbiologically inactive. Whether this substance is intrinsic factor is uncertain since the intrinsic factor activity of

219. Hausmann, K., and Mulli, K.: The Haemopoietic Effect of Vitamin B_{12} Prepared from Fish, Lancet **1**:185, 1952.

220. Pennington, R. J.: A Heat Labile Vitamin B_{12} Complex in Faeces, Biochem. J. **48**:xviii, 1951.

221. Sources of Vitamin B_{12}, editorial, Lancet **1**:197, 1952.

222. Peeler, H. T.; Yacowitz, H.; Carlson, C. W.; Miller, R. F.; Norris, L. C., and Heuser, G. F.: Studies on the Vitamin B_{12} Content of Feedstuffs and Other Materials, J. Nutrition **43**:49, 1951.

223. Collins, R. A.; Harper, A. E.; Schreiber, M., and Elvehjem, C. A.: The Folic Acid and Vitamin B_{12} Content of the Milk of Various Species, J. Nutrition **43**:313, 1951.

224. Ungley, C. C.: Vitamin B_{12}: Part 2. A Review of the Clinical Aspects, Nutrition Abst. & Rev. **21**:1, 1951-1952.

225. Castle, W. B.: Present Status of the Etiology of Pernicious Anemia, Ann. Int. Med. **34**:1093, 1951. Witts, L. J.: Pathogenesis of the Megaloblastic Anaemias, Lancet **2**:367, 1951. Girdwood, R. H.: Vitamin B_{12} and Folic Acid in the Megaloblastic Anaemias, Edinburgh M. J. **58**:309, 1951; The Interrelationships of Factors That Influence the Megaloblastic Anemias, Blood **7**:77, 1952.

226. Hall, B. E.: Studies on the Nature of the Intrinsic Factor of Castle, Brit. M. J. **2**:585, 1950.

227. Bethell, F. H.; Swendseid, M. E.; Miller, S., and Cintron-Rivera, A. A.: Cobalamin (Vitamin B_{12}) and the Intrinsic Factor of Castle, Ann. Int. Med. **35**:518, 1951.

gastric juice, measured clinically, can be destroyed without affecting its ability to combine with vitamin B_{12}.[212] Glass and associates [228] have isolated a "glandular mucoprotein" from human gastric juice which potentiates the effect of vitamin B_{12} administered orally to patients with pernicious anemia. The chemical and physical properties of this substance were similar to those of intrinsic factor. Wolf [229] was unable to find evidence for a chemical reaction between vitamin B_{12} and intrinsic factor which might result in a gross structural change in vitamin B_{12}. On the other hand, Bethell [227] has reported an experiment which suggests that the absorbable form of vitamin B_{12} may be a complex: When vitamin B_{12} bound to intrinsic factor was split by pancreatic enzymes, the material was active microbiologically and more active clinically, on oral administration, than the original vitamin B_{12}, although intrinsic factor had been destroyed and the combination of vitamin B_{12}-intrinsic factor had been split. Callender and Lajtha,[230] in marrow culture studies, found that normal gastric juice and vitamin B_{12} together formed a thermolabile hematopoietic factor which ripens megaloblasts, whereas either substance alone was inactive. These workers also found that normal serum contains a thermolabile factor which ripens megaloblasts in vitro, whereas serum from untreated patients with pernicious anemia had an inhibitory action. They postulated an extragastric, as well as a gastric, source of intrinsic factor. In studies carried out in vivo, Horrigan and associates [231] found that injection of vitamin B_{12} into the bone marrow of one iliac crest produced maturation at the site of injection but not in the other iliac crest. Burkholder [232] presented evidence suggesting that vitamin B_{12} may be removed from the upper digestive tract by microbial flora under conditions of achlorhydria and that intrinsic factor may prevent consumption of vitamin B_{12} by these bacteria.

Clinical studies indicate that only small amounts of orally administered vitamin B_{12} are absorbed either in normal subjects or in patients with pernicious anemia. Single doses of 25 to 400 γ produce little or no hematopoietic response in patients with pernicious anemia.[233] When larger doses are given, findings are variable. Ungley [224] administered 3,000 γ orally to 17 patients: the median hematopoietic response was approximately equivalent to that expected from an injection of 20 to 40 γ; the best response suggested that about 160 γ had been effectively absorbed. Conley and co-workers [234] noted that the response to 5,000 γ of vitamin B_{12} given orally indicated absorption of at least 25 γ.

228. Glass, G. B. J.; Boyd, L. J.; Rubinstein, M. A., and Svigals, C. S.: Relationship of Glandular Mucoprotein from Human Gastric Juice to Castle's Intrinsic Antianemic Factor, Science **115**:101, 1952.

229. Wolf, D. E.; Wood, T. R.; Valiant, J., and Folkers, K.: Vitamin B_{12} and the Intrinsic Factor, Proc. Soc. Exper. Biol. & Med. **73**:15, 1950.

230. Callender, S. T., and Lajtha, L. G.: On the Nature of Castle's Hemopoietic Factor, Blood **6**:1234, 1951.

231. Horrigan, D.; Jarrold, T., and Vilter, R. W.: Direct Action of Vitamin B_{12} upon Human Bone Marrow: The Effect of Instillations of Vitamin B_{12} and Folic Acid into the Bone Marrow as Studied by Nucleic Acid Staining Techniques, J. Clin. Invest. **30**:31, 1951.

232. Burkholder, P. R.: Microbiological Studies on the Intrinsic Factor of Castle, Science **114**:478, 1951.

233. Ungley.[224] Hall.[226] Meyer, L. M.; Sawitsky, A.; Cohen, B. S.; Krim, M., and Fadem, R.: Oral Treatment of Pernicious Anemia with Vitamin B_{12}, Am. J. M. Sc. **220**:604, 1950.

234. Conley, C. L.; Krevans, J. R.; Chow, B. F.; Barrow, C., and Lang, C. A.: Observations on the Absorption, Utilization and Excretion of Vitamin B_{12}, J. Lab. & Clin. Med. **38**:84, 1951.

The urinary excretion of vitamin B_{12} has been studied by several groups of investigators. Register and Sarett [186] found an average excretion of 31 millimicrograms daily in 14 subjects on normal diets. Similar values were obtained in subjects maintained for three days on the following diets: milk, alkaline-ash, low-protein, and high-protein. Higher values were obtained with high-purine diets, but the increase was due to interfering compounds. On the third day of fasting, six subjects excreted an average of 62 millimicrograms daily. Girdwood [235] reported mean excretion of vitamin B_{12} to be 95 millimicrograms daily in 22 patients with pernicious anemia and 126 millimicrograms daily in 10 control subjects. Conley and co-workers [234] found no measurable increase in urinary excretion of vitamin B_{12} in normal subjects or in patients with pernicious anemia following the oral administration of 10,000 γ. Chow [236] reported that neither a single massive oral dose nor the oral administration of vitamin B_{12} for several months resulted in appearance of vitamin B_{12} activity in the urine. A major portion of the ingested vitamin appeared in the feces. Callender and Spray [237] prepared hematopoietically active extracts from feces. The active substance appeared to be vitamin B_{12}, and the amounts excreted were essentially the same in normal subjects and in subjects with pernicious anemia. Chesterman and collaborators [238] found only minute increases in excretion of vitamin B_{12} of 0.03 to 1 γ after oral doses of 1 to 9 mg. After intramuscular injection of 5 to 1,000 γ of vitamin B_{12} these investigators found that the relation between the dose given and the amount excreted could be expressed as follows: Excretion $=$ Dose -1.2 Dose$^{0.89}$. Sokoloff and associates [239] reported an increase in the percentage excretion of a 30- to 40-γ dose of vitamin B_{12} when this dose was given after administration of large amounts of the vitamin; i. e., there was evidence of saturation. Chesterman and co-workers [238] found that patients with pernicious anemia excreted less vitamin B_{12} after a 20-γ dose than did normal subjects and that continued daily injection did not increase excretion to the extent noted in normal subjects. Boxer and Rickards,[216] using a chemical method, reported prompt urinary excretion of 90 to 95% of a 1.08-mg. dose of vitamin B_{12} given subcutaneously.

Ross [240] developed a method for assaying vitamin B_{12} in body fluids with the use of Euglena gracilis. With this procedure the vitamin B_{12} activity of human sera amounted to 350 to 750 micromicrograms per milliliter. Heating of the sera was necessary to obtain maximal activity, suggesting that vitamin B_{12} circulates in altered or combined form. After injection of vitamin B_{12} the serum levels increased. Chow,[236] using another method, observed an increase in vitamin B_{12} in plasma following intravenous injection of this vitamin.

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235. Girdwood, R. H., and Carmichael, K. M.: The Content of Haemopoietic Factors in Liver Extracts: Relationship to Clinical Response, Brit. M. J. **2**:1357, 1950.

236. Chow, B. F.: Sequelae to the Administration of Vitamin B_{12} to Humans, J. Nutrition **43**:323, 1951.

237. Callender, S. T. E., and Spray, G. H.: Preparation of Haemopoietically Active Extracts from Faeces, Lancet **1**:1391, 1951.

238. Chesterman, D. C.; Cuthbertson, W. F. J., and Pegler, H. F.: Vitamin B_{12} Excretion Studies, Biochem. J. **48**:li, 1951.

239. Sokoloff, M. F.; Sanneman, E. H., Jr., and Beard, M. F.: Urinary Excretion of Vitamin B_{12}, Blood **7**:243, 1952.

240. Ross, G. I. M.: Vitamin B_{12} Assay in Body Fluids, Nature, London **166**:270, 1951.

An excellent summary of the probable fate of ingested and injected vitamin B_{12} has been given by Ungley.[224] He suggests that, although nothing of the actual mechanism of absorption is known, presumably the vitamin combines with a thermolabile substance in the intestinal wall, perhaps the "extragastric intrinsic factor of Lajtha," which may be formed as required, as is postulated for apoferritin.

No vitamin B_{12} was detected, microbiologically, in the tissues of a patient with untreated pernicious anemia who died of coronary thrombosis.[241] However, skin biopsy specimens from three patients with pernicious anemia in relapse exhibited vitamin B_{12} activity.

Girdwood [235] has discussed the amounts of hematopoietic factors in liver extract in relation to clinical response. The effectiveness of vitamin B_{12} as maintenance therapy in pernicious anemia has been reported by several investigators.[242] Conley and Krevans [243] encountered five patients with the neurological manifestations of pernicious anemia but with little or no anemia. These patients had been receiving multivitamin preparations containing folic acid, presumably in amounts sufficient to produce hematologic remission. Treatment with vitamin B_{12} brought about neurological improvement. It seems likely that this situation will be noted with increasing frequency in view of the large number of vitamin products which contain folic acid.

A number of reports have dealt with the use of vitamin B_{12} in macrocytic anemia other than pernicious anemia. In a patient with megaloblastic anemia following total gastrectomy, administration of vitamin B_{12} led to suboptimal response, while addition of folic acid induced complete hematologic remission.[244] Chaudhuri [245] reported successful treatment with vitamin B_{12} in 16 cases of megaloblastic anemia of pregnancy and a good response to this vitamin in 4 of 5 cases of tropical macrocytic anemia. The fifth patient responded to folic acid. Findings indicated that larger doses of vitamin B_{12} are required in these anemias than in pernicious anemia. Thompson and Ungley [246] found that few patients with megaloblastic anemia of pregnancy responded to liver extract or vitamin B_{12} in doses more than adequate for the treatment of pernicious anemia, whereas response to folic acid was satisfactory. Holly [247] reported beneficial effects from the addition of ascorbic acid to vitamin B_{12} therapy in megaloblastic anemia of pregnancy.

241. Girdwood, R. H.: The Content of Haemopoietic Factors in Some Human Tissues, Brit. J. Nutrition **5**:xi, 1951.

242. Campbell, D. C., and Hall, B. E.: Maintenance Therapy with Vitamin B_{12} for Patients with Pernicious Anemia, J. Lab. & Clin. Med. **38**:797, 1951. Beard, M. F.; McIlvanie, S. K., and Nataro, M.: Vitamin B_{12} in Pernicious Anemia in Remission, South. M. J. **43**:678, 1950. Jonsson, U., and Rundles, R. W.: Vitamin B_{12} and Pteroylglutamic Acid Deficiency in Macrocytic Anemia, Am. J. Med. **9**:401, 1950.

243. Conley, C. L., and Krevans, J. R.: Development of Neurologic Manifestations of Pernicious Anemia During Multivitamin Therapy, New England J. Med. **245**:529, 1951.

244. Conway, N. S., and Conway, H.: Vitamin B_{12} and Folic Acid in Megaloblastic Anaemia After Total Gastrectomy, Brit. M. J. **1**:158, 1951.

245. Chaudhuri, S.: Vitamin B_{12} in Megaloblastic Anaemia of Pregnancy and Tropical Nutritional Macrocytic Anaemia, Brit. M. J. **2**:825, 1951.

246. Thompson, R. B., and Ungley, C. C.: Megaloblastic Anemia of Pregnancy and the Puerperium, Quart. J. Med. **20**:187, 1951.

247. Holly, R. G.: Megaloblastic Anemia in Pregnancy: Remission Following Combined Therapy with Ascorbic Acid and Vitamin B_{12}, Proc. Soc. Exper. Biol. & Med. **78**:238, 1951.

Diez-Rivas and collaborators [248] observed that in tropical sprue vitamin B_{12} administered orally, either with or without gastric juice, had less hematopoietic effect than smaller amounts of B_{12} given parenterally or than folic acid given orally. The minimal oral dose of vitamin B_{12} which initiated appreciable reticulocytosis and definite clinical improvement was 150 to 200 γ daily. Gastric juice potentiated the hematopoietic effect of vitamin B_{12} in some instances. Youmans [249] reported that vitamin B_{12} was effective in relieving glossitis, stomatitis, and slight impairment in absorption from the intestinal tract in several patients without anemia. The possibility was considered that findings in these patients may represent the earliest stage of the sprue syndrome.

Two cases of megaloblastic anemia responding to crystalline penicillin G have been reported.[250] It is postulated that the hematopoietic effect of penicillin is due to an influence on bacterial synthesis or metabolism in the intestinal tract, removal of some hematopoietic antagonist, or an effect on the synthesis of nucleoproteins. Aureomycin has also been reported to stimulate hematopoiesis.[251] Findings of Davis and Chow [252] are of interest in this regard. By incorporating radioactive cobalt into the diet of rats, it was shown that aureomycin caused an increase in activity, both radioactive and microbiological, of the vitamin B_{12} content of feces.

Vitamin B_{12} has been shown to be essential for growth in many species of animals.[253] Findings in man are equivocal to date. Addition of vitamin B_{12} to the diet of premature or full-term newborn infants had no significant effect on gain in weight or increase in length.[254] Administration of vitamin B_{12} to chronically ill and to clinically healthy children was followed by a greater gain in weight than in corresponding control groups of children [236]; results were considered to be suggestive of trends which need confirmation.

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248. Diez-Rivas, F.; Suarez, R. M.; Morales, F. H., and Santiago, E. P.: The Oral Administration of Vitamin B_{12} in Tropical Sprue, Ann. Int. Med. **36**:583, 1952.

249. Youmans, J. B.: Glossitis and Stomatitis Without Anemia Responding to Folic Acid and Vitamin B_{12}, Tr. A. Am. Physicians **64**:304, 1951.

250. Foy, H.; Kondi, A., and Hargreaves, A.: The Response of Megaloblastic Macrocytic Anaemia to Crystalline Penicillin G, Preliminary Report, Brit. M. J. **1**:380, 1951; Response of Megaloblastic Anaemia of Pregnancy to Crystalline Penicillin G, ibid. **1**:1108, 1951.

251. Lichtman, H.; Ginsberg, V., and Watson, J.: Therapeutic Effect of Aureomycin in Pernicious Anemia, Proc. Soc. Exper. Biol. & Med. **74**:884, 1950.

252. Davis, R. L., and Chow, B. F.: Content of Radioactive Vitamin B_{12} in Feces of Rats Fed Co^{60} and Aureomycin, Proc. Soc. Exper. Biol. & Med. **77**:218, 1951.

253. Johnson, B. C.; Neumann, A. L.; Nesheim, R. O.; James, M. F.; Krider, J. L.; Dana, A. S., and Thiersch, J. B.: The Interrelationship of Vitamin B_{12} and Folic Acid in the Baby Pig, J. Lab. & Clin. Med. **36**:537, 1950. Cuthbertson, W. F. J., and Thornton, D. M.: Effect of Parental Nutrition on the Growth Response of the Rat to Vitamin B_{12}, Brit. J. Nutrition **5**:xii, 1951. Coates, M. E.; Harrison, G. F.; Kon, S. K.; Mann, M. E., and Rose, C. D.: Effect of Antibiotics and Vitamin B_{12} on Growth of Normal and "Animal Protein Factor" Deficient Chicks, Biochem. J. **48**:xiii, 1951.

254. Chinnock, R. F., and Rosenberg, H. W.: Results of Administration of Vitamin B_{12} to Newborn Infants, J. Pediat. **40**:182, 1952. Rascoff, H.; Dunewitz, A., and Norton, R.: The Weight Progress of Premature Infants Given Supplementary Feedings of Vitamin B_{12}: A Comparative Study, ibid. **39**:61, 1951. Mitchell, T.; Etteldorf, J. N.; Tuttle, A. H., and Clayton, G. W., Jr.: Crystalline Vitamin B_{12} and Sodium Folvate in Prematurity, Pediatrics **8**:821, 1951.

Sancetta and co-workers [255] obtained improvement in neurological manifestations of diabetes mellitus in 10 of 12 patients following administration of vitamin B_{12} in amounts of 15 to 30 γ daily at first, then once or twice weekly. Massive doses, such as 900 to 1,000 γ daily, as previously suggested, do not seem essential except perhaps in an occasional patient. Further trial of vitamin B_{12} in the treatment of neurological abnormalities in diabetes seems well worth while.

Vitamin B_{12} has been reported to be beneficial in the treatment of a number of diverse conditions; lupus erythematosus,[256] herpes zoster,[257] chronic allergic dermatoses,[258] chronic ulcerative colitis,[259] and osteoarthritis.[260] Evaluation of such therapy must await further controlled study.

In animal studies, vitamin B_{12} has been shown to have a number of important functions, in addition to those related to growth and hematopoiesis. In most instances, application to human nutrition remains for the future. In rats, vitamin B_{12} appears to be a factor in the prevention of hydrocephalus,[261] to influence reproduction and lactation,[262] and to have a sparing action on protein when hyperthyroidism has been induced.[263]

The lipotropic effects of vitamin B_{12} and interrelationships between this vitamin, folic acid, choline, and methionine have been discussed in the section on nutrition in liver disease.

SPRUE

Several authors have reported beneficial results from the use of cortisone or corticotropin (adrenocorticotropic hormone) in the therapy of the sprue syndrome.[264] Badenoch [265] was unable to control steatorrhea or to demonstrate

255. Sancetta, S. M.; Ayres, P. R., and Scott, R. W.: The Use of Vitamin B_{12} in the Management of the Neurologic Manifestations of Diabetes Mellitus with Notes on the Administration of Massive Doses, Ann. Int. Med. **35**:1028, 1951.

256. Goldblatt, S.: Treatment of Lupus Erythematosus with Vitamin B_{12}: Preliminary Report on 4 Cases, J. Invest. Dermat. **17**:303, 1951.

257. Heyblon, R.: Traitement du zona par la vitamine B_{12}, Méd. franç. **11**:102, 1951.

258. Simon, S. W.: Vitamin B_{12} Therapy in Allergy and Chronic Dermatoses, J. Allergy **22**:183, 1951. Dieterich, J. P.: Infantile Eczema: Therapy with Vitamin B_{12}, Ann. West. Med. & Surg. **5**:47, 1951.

259. Rail, G. A.: Two Cases of Ulcerative Colitis Treated with Vitamin B_{12}, Lancet **2**:816, 1951.

260. Hallahan, J. D.: Symptomatic Relief of Osteo-Arthritis and Osteoporosis with Vitamin B_{12}: Preliminary Report, Am. Pract. & Digest. Treat. **3**:27, 1952.

261. O'Dell, B. L.; Whitley, J. R., and Hogan, A. G.: Vitamin B_{12}, a Factor in Prevention of Hydrocephalus in Infant Rats, Proc. Soc. Exper. Biol. & Med. **76**:349, 1951.

262. Sure, B.: Vitamin B_{12} in Reproduction and Lactation, J. Am. Dietet. A. **27**:564, 1951.

263. Rupp, J.; Paschkis, K. E., and Cantarow, A.: Influence of Vitamin B_{12} and Liver Extract on Nitrogen Balance of Normal and Hyperthyroid Rats, Proc. Soc. Exper. Biol. & Med. **76**:432, 1951.

264. Almy, T. P.: A Refractory Case of Idiopathic Steatorrhea (Non-Tropical Sprue) with Observations on the Therapeutic Effect of Salt-Poor Human Albumin and of the Adrenocorticotropic Hormone, Ann. Int. Med. **34**:1041, 1951. Adlersberg, D.; Colcher, H., and Drachman, S. R.: Studies on the Effect of Cortisone and Pituitary Adrenocorticotropic Hormone (ACTH) in the Sprue Syndrome, Gastroenterology **19**:674, 1951. Taylor, A. B.; Wollaeger, E. E.; Comfort, M. W., and Power, M. H.: The Effect of Cortisone on Non-Tropical Sprue (Idiopathic Steatorrhea), ibid. **20**:203, 1952.

265. Badenoch, J.: Cortisone in Idiopathic Steatorrhea, Brit. M. J. **1**:356, 1952; Results of Protracted Treatment of Idiopathic Steatorrhea, Lancet **1**:238, 1952.

significant reduction in the fecal excretion of fat in four patients treated with cortisone, although seven patients treated with folic acid and dietary regulation responded well. Suárez [266] has reported that more than 100 patients with sprue have been maintained in clinical and hematologic remission over a five-year period with 2.5 mg. of folic acid daily. An additional 20 patients have been maintained in remission for almost two years with weekly injections of 15 γ of vitamin B_{12}. Simultaneous oral administration of 10 γ of vitamin B_{12} and 0.67 mg. of folic acid has produced suboptimal results. Wollaeger and Scribner [267] have described delayed excretion of water with regular nocturnal diuresis as a characteristic finding in the sprue syndrome. They suggest that large volumes of water are retained in the small intestine during an abnormally prolonged period of digestion and absorption of food, and that when absorption is finally complete, the retained water is excreted. A report of the successful use of polysorbate 80 ("sorlate"), an emulsifying agent, in the treatment of two children with celiac disease suggests its possible usefulness in the management of the sprue syndrome and other conditions associated with defective absorption of fat.[268]

CHOLINE

Reference has been made to choline in its interrelationships with other lipotropic substances elsewhere in this review. Arnstein and Neuberger [269] have presented evidence that vitamin B_{12} aids the conversion of glycine to choline in rats. Interesting studies on the absorption and excretion of choline in normal subjects and in patients with hepatobiliary diseases have been reported by de la Huerga and Popper.[270] They found that after oral administration of 2 to 8 gm. of choline base very small amounts of choline were excreted in the urine, but that approximately two-thirds of the choline nitrogen ingested was excreted as trimethylamine and its oxide, a substance with no lipotropic activity. Incubated stool dilutions readily transformed choline to trimethylamine. Excretion of trimethylamine oxide in the urine following oral administration of choline could be reduced by previous administration of aureomycin or phthalylsulfathiazole (sulfathalidine®). Intravenous administration of choline did not increase urinary trimethylamine excretion, suggesting that the greatest portion of trimethylamine originates in the intestine from ingested choline and not from transformation in the tissues.

When choline was administered intravenously, approximately 9% of the total dose was excreted in the urine by normal subjects and patients with cirrhosis,

266. Suárez, R.: Foreign Newsletter—Puerto Rico, Blood **6**:1293, 1951.

267. Wollaeger, E. E., and Scribner, B. H.: Delayed Excretion of Water with Regular Nocturnal Diuresis in Patients with Nontropical Sprue (Idiopathic Steatorrhea), Gastroenterology **19**:224, 1951.

268. Boyd, H., and Helfrick, F.: Celiac Disease Treated with Polyoxyethylene Sorbitan Monooleate, J. Pediat. **38**:493, 1951.

269. Arnstein, H. R. V., and Neuberger, A.: The Effect of Vitamin B_{12} on the Conversion of Glycine to Choline, Biochem. J. **48**:ii, 1951.

270. (*a*) de la Huerga, J., and Popper, H.: Urinary Excretion of Choline Metabolites Following Choline Administration in Normals and Patients with Hepatobiliary Diseases, J. Clin. Invest. **30**:463, 1951. (*b*) Popper, H., in discussion at Conference on Liver Injury, New York, May 21-22, 1951, in Transactions of the 10th Conference, New York, Josiah Macy, Jr., Foundation, 1951, p. 124. (*c*) de la Huerga, J.; Popper, H., and Steigmann, F.: Urinary Excretion of Choline and Trimethylamines After Intravenous Administration of Choline in Liver Disease, J. Lab. & Clin. Med. **38**:904, 1951.

whereas twice this amount was excreted by patients with acute hepatitis.[270c] These findings were tentatively explained as the result of (1) reduced utilization of choline in liver disease and (2) increased demand as a result of choline deficiency in cirrhosis. The authors stated that the intravenous administration of 1 to 2 gm. of choline base in 500 to 1,000 ml. of solution has been found safe for therapeutic purposes.

INOSITOL

Best and his associates [271] have demonstrated that inositol exhibits lipotropic activity in animals only when added to a hypolipotropic diet devoid of fat and exerts either no or, at the most, a very slight effect when fat is added to the diet. Parenteral administration of inositol did not enhance its activity. Reasons for confusion in the literature concerning the lipotropic potency of inositol are discussed.

ASCORBIC ACID

Although ascorbic acid has been available since 1932, the demonstration by Sealock and Goodland [272] that it is a necessary coenzyme in the metabolic oxidation of the amino acid tyrosine is the first adequate explanation of the chemistry of one of its biological functions. The relation of ascorbic acid to the functional activity of the adrenal cortex has been studied extensively and is the subject of a comprehensive review by Pirani.[273] Stimulation of the adrenal cortex leads to depletion of ascorbic acid stores in this gland, although adrenal cortical function is maintained even when depletion is severe. Increased utilization and requirement of ascorbic acid are observed under conditions of stress and to a less marked extent after the administration of corticotropin. Administration of ascorbic acid seems indicated in chronic stress, particularly after burns or other trauma, and during prolonged therapy with corticotropin. The function of ascorbic acid in the adrenal cortex remains unknown and may be of "a nonspecific nature related to respiratory activity and metabolic rate." [273] Reppert and associates [274] have studied the effects of ascorbic acid upon the hyaluronidase-hyaluronic acid reaction in vitro and in vivo and suggest that in some cases in which capillary fragility is corrected by ascorbic acid the effect may be due to inhibition of this system. Moore and Dubach [275] have found that ascorbic acid fed with food into which radioactive iron has been incorporated causes increased absorption of iron from the intestinal tract. El Ridi [276] reported the use of *peri*naphthindanetrione hydrate as a new reagent for the estima-

271. Best, C. H.; Ridout, J. H.; Patterson, J. M., and Lucas, C. C.: A Statistical Evaluation of the Lipotropic Action of Inositol, Biochem. J. **48**:448, 1951. Best, C. H.; Lucas, C. C.; Patterson, J. M., and Ridout, J. H.: The Rates of Lipotropic Action of Choline and Inositol Under Special Dietary Conditions, ibid. **48**:452, 1951.

272. Sealock, R. R., and Goodland, R. L.: Ascorbic Acid, a Coenzyme in Tyrosine Oxidation, Science **114**:645, 1951.

273. Pirani, C. L.: Review: Relation of Vitamin C to Adrenocortical Function and Stress Phenomena, Metabolism **1**:197, 1952.

274. Reppert, E.; Donegan, J., and Hines, L. E.: Ascorbic Acid and the Hyaluronidase-Hyaluronic Acid Reaction, Proc. Soc. Exper. Biol. & Med. **77**:318, 1951.

275. Moore, C. V., and Dubach, R.: Observations on the Absorption of Iron from Foods Tagged with Radioiron, Tr. A. Am. Physicians **64**:245, 1951.

276. El Ridi, M. S.; Moubasher, R., and Hassan, Z. F.: Spectrophotometric Assay of Ascorbic Acid in Pure Solutions, Blood Plasma and Urine with periNaphthindanetrione Hydrate, Biochem. J. **49**:246, 1951.

tion of ascorbic acid in pure solutions and body fluids. Freeman and Hafkespring [277] have made a comparative study of ascorbic acid levels in blood, urine, saliva, and gastric secretion.

Sigurjonsson [278] has measured the excretion of vitamin C in the urine following the administration of oral test doses and has confirmed the previous finding that considerable losses of this vitamin occur, through either destruction or incomplete absorption, when large test amounts are administered. Chen and Schuck [279] have studied the urinary excretion of reduced ascorbic acid, dehydroascorbic acid, and diketogulonic acid by subjects on varied diets with and without supplementation with crystalline vitamin C, cantaloupe, or grapefruit. Holmes [280] has pointed out that the ascorbic acid content of orange juice is highly variable and suggests the use of crystalline vitamin C to provide a standardized uniform amount in infant feeding. Holley and McLester [281] have reported the appearance of a hemorrhagic tendency, typical of vitamin C deficiency, in two patients during the course of prolonged administration of corticotropin. Dao and Shank [282] have found that intravenous administration of 1 gm. of ascorbic acid daily to surgical patients during the preoperative and postoperative periods prevents the decline in plasma ascorbic acid concentration which was observed postoperatively in an untreated control group. An increase in the urinary excretion of steroids was observed in the treated patients in the postoperative period. Klasson [283] reported that the use of ascorbic acid topically, orally, or parenterally reduced the time interval necessary for skin grafting in 62 cases of mild to severe burns so treated. Stern [284] has observed that large doses of vitamin C are effective in the treatment of prickly heat, despite the absence of any clinical evidence of vitamin C deficiency.

VITAMIN D

An outstanding contribution to knowledge of vitamin metabolism has been made by Zetterström and his collaborators,[285] who have studied the role of vitamin D in enzymatic reactions. Utilizing a water-soluble phosphorylated vitamin D_2, they have demonstrated that vitamin D activates alkaline phosphatases of bone, kidneys, and intestine, this effect being particularly marked in the case of bone phosphatase. Thus, an enzymatic explanation of the role of vitamin D in the prevention of rickets

277. Freeman, J. T., and Hafkespring, R.: Comparative Study of Ascorbic Acid Levels in Gastric Secretion, Blood, Urine and Saliva, Gastroenterology **18**:224, 1951.

278. Sigurjonsson, J.: Excretion of Vitamin C in Urine Following Repeated Administration of Big Test Doses, Brit. J. Nutrition **5**:216, 1951.

279. Chen, S. D., and Schuck, C.: Excretion of the 2 Biologically Active Forms of Ascorbic Acid and Diketogulonic Acid by Human Subjects, J. Nutrition **45**:165, 1951.

280. Holmes, A. D.; Jones, C. P., and Tripp, E.: Use of Ascorbic Acid Tablets to Enrich Milk for Infant Feeding, J. Pediat. **39**:320, 1951.

281. Holley, H. L., and McLester, J. S.: Manifestations of Ascorbic Acid Deficiency After Prolonged Corticotropin Administration, A. M. A. Arch. Int. Med. **88**:760, 1951.

282. Dao, T. L., and Shank, R. E.: Urinary Excretion of Adrenocortical Hormones in Surgical Patients Provided Supplements of Ascorbic Acid, J. Lab. & Clin. Med. **38**:802, 1951.

283. Klasson, D. H.: Ascorbic Acid in the Treatment of Burns, New York J. Med. **51**:2388, 1951.

284. Stern, R. L.: Relief of Prickly Heat with Vitamin C, J. A. M. A. **145**:175, 1951.

285. Zetterström, R., and Ljunggren, M.: Activation of Alkaline Phosphatase from Different Organs by Phosphorylated Vitamin D_2, Acta chem. scandinav. **5**:283, 1951; Activation of Aerobic Oxidation in Kidney Mitochondria by Phosphorylated Vitamin D_2, ibid. **5**:343, 1951.

is provided. Alkaline phosphatases are believed to have a role in intestinal absorption, renal tubular reabsorption, and the deposition of phosphate in bone. If these phosphatases need to be activated by vitamin D, a deficiency of this vitamin will result in poor intestinal absorption, excessive renal loss of phosphates, and poor ossification, the three essential aspects of rickets.

Green [286] has published a series of detailed studies on methods of analysis of vitamin D. Snyder and associates [287] have proposed a new technique for bioassay of vitamin D, based upon the parenteral injection of radioactive phosphorus into rats maintained on a rachitogenic diet.

Pedersen and McCarroll [288] have investigated the occurrence of resistant rickets in children undergoing corrective surgical procedures for bone deformities and in the families of these children. A high familial incidence of the disease was noted. In adults, short stature due to poor growth of the lower extremities was the outstanding feature, and a number had shown progression of the disease after the age of epiphyseal closure. In the children, epiphyseal line improvement in all visible epiphyses was noted on roentgenograms during postoperative immobilization in plaster without the administration of vitamin D. Large doses of vitamin D produced toxic symptoms in immobilized patients but were well tolerated by the same subjects when ambulatory.

Chaplin and associates [289] have reviewed the clinical features of 111 cases of vitamin D intoxication previously reported in the literature and have added a detailed report on 7 additional cases. Adams [290] has reported a case of vitamin D intoxication and pointed out the necessity of considering overdosage of this vitamin as a possible cause of uremia and hypercalcemia.

VITAMIN E

No clinical evidence of vitamin E deficiency has been established in man as yet. Animal experiments have suggested that tocopherol may play a role in the alleviation or prevention of toxic liver damage under certain conditions. Studies by Klatskin and associates [291] have demonstrated that fasting plasma tocopherol levels

286. Green, J.: Studies on the Analysis of Vitamins D: I. The Reaction of Vitamins D and Related Substances with Iodine Trichloride, Biochem. J. **49**:36, 1951; II. The Analytical Purification of Vitamin D by Differential Solubility, Precipitation Reactions and Chromatography, ibid. **49**:45, 1951; III. Experiments on the Separation of Vitamin D from Vitamin A and Other Substances by Condensation Techniques and Selective Irradiation, ibid. **49**:54, 1951; IV. Studies on the Irradiation of Ergosterol and 7-Dehydrocholesterol and the Analysis of the Products for Cholesterol, Vitamin D_3 and Component Sterols, ibid. **49**:232, 1951; V. The Chemical Determination of Vitamin D in Fish Liver Oils and Other Materials Containing Vitamin A, ibid. **49**:243, 1951.

287. Snyder, R. H.; Eisner, H. J., and Steenbock, H.: The Determination of Vitamin D with Radiophosphorus, J. Nutrition **45**:305, 1951.

288. Pedersen, H. E., and McCarroll, H. R.: Vitamin-Resistant Rickets, J. Bone & Joint Surg. **33-A**:203, 1951.

289. Chaplin, H. J.; Clark, L. D., and Ropes, M. W.: Vitamin D Intoxication, Am. J. M. Sc. **221**:369, 1951.

290. Adams, F. D.: Reversible Uremia with Hypercalcemia Due to Vitamin D Intoxication, New England J. Med. **244**:590, 1951.

291. Klatskin, G., and Krehl, W. A.: The Significance of the Plasma Tocopherol Concentration and of Tocopherol Tolerance Tests in Liver Disease, J. Clin. Invest. **29**:1528, 1950. Klatskin, G., and Molander, D. W.: The Absorption and Excretion of Tocopherol in Laennec's Cirrhosis, ibid. **31**:159, 1952.

were significantly lower in a group of patients with liver disease than in a group of healthy controls, but did not differ from the levels obtained in a series of hospitalized patients without evidence of hepatic disorders. The level of plasma tocopherol found in the group with liver disease could not be correlated with the type of disease present, extent of dysfunction as estimated clinically, or the level of serum cholesterol. Patients with liver disease showed less of an increase in plasma tocopherol levels after administration of an oral dose of 500 mg. than did normal subjects, but saturation and balance studies failed to reveal any evidence of impaired absorption of tocopherol. The authors suggested that the findings in liver disease were manifestations of tissue depletion of vitamin E secondary to deficient dietary intake.

Wright, Filer, and Mason [292] have studied serum tocopherol levels in premature and full-term infants and their mothers and concluded that placental transfer of vitamin E is limited, while transfer in the milk is much more extensive. The attainment of normal levels of serum tocopherol in bottle-fed infants was considerably slower than in breast-fed children.

Crump and Heiskell [293] have reported additional observations upon the use of alphatocopherol and calcium gluconate in the prevention of thromboembolic disease. Levitan [294] was unable to demonstrate any beneficial effect of vitamin E, either alone or in combination with rutin and ascorbic acid, upon capillary fragility in patients with diabetic retinopathy. Weisstub [295] reports the use of vitamin E and testosterone in the treatment of dermatomyositis, and Van Duzen and Mustain [296] report the alleviation of symptoms in six patients with interstitial cystitis after prolonged administration of alphatocopherol.

VITAMIN K

An interesting study of the factors concerned in the normal clotting mechanism has been reported by Sørbye and his associates.[297] They have succeeded in isolating from the plasma of vitamin K-deficient chicks a protein factor, designated the "kappa factor," which is capable of reducing the plasma prothrombin time of bishydroxycoumarin (dicumarol®)-treated animals, but is without effect upon the prothrombin time in vitamin K deficiency. Similarly, a second factor, the "delta factor," isolated from the plasma of chicks treated with bishydroxycoumarin, was capable of reducing the prothrombin time of vitamin K-deficient chick plasma, but not that of plasma from chicks given bishydroxycoumarin therapy. From these results they postulated that at least four factors are necessary for normal clotting:

292. Wright, S. W.; Filer, L. J., and Mason, K. E.: Vitamin E Levels in Infants and Mothers, Pediatrics **7**:386, 1951.

293. Crump, W. E., and Heiskell, E. F., Jr.: Prevention of Thromboembolism by Alpha-Tocopherol and Calcium Gluconate, Texas J. Med. **48**:11, 1952.

294. Levitan, B. A.: Clinical Observations on the Effects of Injectable Rutin, Esculin, Adrenoxyl, and Vitamin E on the Capillary Fragility of Diabetic Retinopathy, Am. J. M. Sc. **221**:185, 1951.

295. Weisstub, I.: A Case of Dermatomyositis, Canad. M. A. J. **64**:342, 1951.

296. Van Duzen, R. E., and Mustain, R.: Alpha-Tocopherol in Treatment of Interstitial Cystitis: Preliminary Report, J. Urol. **65**:1033, 1951.

297. Sørbye, Ø.; Kruse, I., and Dam. H.: The Factor in Vitamin K Deficient Plasma Which Accelerates the Coagulation of Dicumarol Plasma, Acta chem. Scandinav. **4**:549, 1950; The Factor in Dicumarol Plasma Which Accelerates the Coagulation of Plasma from Vitamin K Deficient Chicks, ibid. **4**:831, 1950.

prothrombin proper, the "kappa" and "delta factors," and the labile factor of fresh plasma or V factor. Deficiency of vitamin K apparently produces a defect in the "delta factor," whereas bishydroxycoumarin produces a deficiency of the "kappa factor."

Quick and Collentine [298] have demonstrated that vitamin K_1 is more effective than menadione in restoring the prothrombin time of dogs rendered deficient in vitamin K by cholecystnephrostomy. They postulated that both vitamin K and menadione can form a prosthetic group which unites with an apoenzyme responsible for the synthesis of prothrombin, and that menadione is a less efficient prosthetic group than vitamin K_1. Douglas and Brown [299] feel that vitamin K_1 is the most reliable and efficient antidote for the treatment of hypoprothrombinemia produced by bishydroxycoumarin or ethyl biscoumacetate ("tromexan"). Ley and associates [300] have reported that vitamin K_1 in large doses restored the prothrombin time to normal in a case of idiopathic hypoprothrombinemia after 1,000 mg. of menadione had been given without success. Stare and associates [301] have succeeded in preparing an emulsion of vitamin K_1 for intravenous use and have reported its effect upon bishydroxycoumarin-induced hypoprothrombinemia in dogs and man. The emulsion is apparently stable for a reasonably long period, is free of undesirable side-effects, and has a more rapid action than do other preparations of the vitamin.

MINERAL NUTRITION

The numerous recent studies of problems of water and electrolyte balance in various clinical conditions cannot be reviewed here.[302] Much attention has been

298. Quick, A. J., and Collentine, G. E.: The Role of Vitamin K in the Formation of Prothrombin, J. Lab. & Clin. Med. **36**:976, 1950. Collentine, G. E., and Quick, A. J.: The Interrelationship of Vitamin K and Dicoumarin, Am. J. M. Sc. **222**:7, 1951.

299. Douglas, A. S., and Brown, A.: Effect of Vitamin-K Preparations on Hypoprothrombinaemia Induced by Dicumarol and Tromexan, Brit. M. J. **1**:412, 1952.

300. Ley, A. B.; Reader, G. G.; Sorevson, C. W., and Overman, R. S.: Idiopathic Hypoprothrombinemia Associated with Hemorrhagic Diathesis, and the Effect of Vitamin K, Blood **6**:750, 1951.

301. Shoshkes, M.; Geyer, R. P.; Yee, G. S., and Stare, F. J.: The Treatment of Dicumarol-Induced Hypoprothrombinemia in Dogs with Emulsified Vitamin K_1 Administered Intravenously, J. Lab. & Clin. Med. **36**:531, 1950. Watkin, D. M.; Van Itallie, T. B.; Logan, W. B.; Geyer, R. P.; Davidson, C. S., and Stare, F. J.: The Treatment of Dicumarol-Induced Hypoprothrombinemia in Man with Emulsified Vitamin K_1 Administered Intravenously, ibid. **37**:269, 1951. Van Itallie, T. B.; Geyer, R. P., and Stare, F. J.: Vitamin K_1 Emulsions in Bishydroxycoumarin Emergencies: Results of Intravenous Administration, J. A. M. A. **147**:1652, 1951.

302. Fraser and Flink.[174] Orloff, J.; Welt, L. G., and Stowe, L.: The Effects of Concentrated Salt-Poor Albumin on the Metabolism and Excretion of Water and Electrolytes, J. Clin. Invest. **29**:770, 1950. Lewin, K. K., and Criep, L. H.: The Electrocardiogram in Potassium Disturbance, Postgrad. Med. **8**:345, 1950. Manter, W. B.: Practical Potassium Therapy, J. Maine M. A. **41**:453, 1950. Mateer, F. M.; Greenman, L.; Austin, A. C.; Peters, J. H.; Mermelstein, H.; Weigand, F., and Danowski, T. S.: The Use of Sodium Chloride in the Pre-Operative Treatment of Pyloric Stenosis in Infants, Am. J. M. Sc. **221**:21, 1951. Elkenton, J. R.; Squires, R. D., and Crosley, H. P.: Intracellular Cation Exchanges in Metabolic Alkalosis, J. Clin. Invest. **30**:369, 1951. Campbell, D. A.; Hay, K. M., and Tonks, E. M.: An Investigation of the Salt and Water Balance in Migraine, Brit. M. J. **2**:1424, 1951. Dieckmann, W. J.; Pottinger, R.; Rynkiewicz, L.; Segal, P., and Mathews, J.: Etiology of Pre-Eclampsia-Eclampsia: IV. Sodium Chloride Test for the Diagnosis of Pre-Eclampsia, J. Lab. & Clin. Med.

directed to the causation and management of potassium deficiency. Some aspects of low-sodium diets in the therapy of hypertension and in the control of ascites due to liver disease have been considered in the preceding sections of this paper. The role of the liver in the control of fluid and electrolyte balance has been comprehensively and critically reviewed by Snavely.[303] An excellent discussion of present concepts of the role of sodium in disease states has been presented by Danowski [304] and should be of considerable interest to clinicians.

Forbes and Perley [305] have utilized the isotope dilution technique to estimate total body sodium content and to study changes associated with normal growth. They estimate that at least 82% of total body sodium is present as "exchangeable sodium," which is most intimately concerned with fluid balance. The determination of the specific activity of sodium in bone suggests that as much as 60% of skeletal sodium may be metabolically inert.

A new approach to the management of disorders associated with the retention of electrolytes in body fluids has been offered by the development of the carboxylic cation-exchange resins. An excellent symposium has been presented by Danowski and his colleagues,[306] which considers in detail the clinical and biochemical effects

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38:803, 1951. Reimers, W. L., and Zollinger, R. M.: Urinary Chloride Determinations in the Estimation of the Salt Requirement in Surgical Patients, A. M. A. Arch. Surg. **63**:70, 1951. Wilhelm, S. K.: Alterations in Serum Potassium and Sodium in Acute Myocardial Infarction, Am. J. Clin. Path. **21**:146, 1951. Elliott, H. C., and Holley, H. L.: Serum Sodium and Potassium Values in 400 Normal Human Subjects, Determined by the Beckman Flame Photometer, ibid. **21**:831, 1951. Sunderman, F. W.: Studies in Serum Electrolytes: XVII. Some Clinical Aspects, ibid. **21**:319, 1951. Schoch, H. K.: Potassium Deficiency in Chronic Renal Disease, A. M. A. Arch. Int. Med. **88**:20, 1951. Cole, W. H.: Laboratory Aids in Surgery, with Special Reference to Potassium Deficiency, A. M. A. Arch. Surg. **62**:737, 1951. Lans, H. S.; Stein, I. F., Jr.; Becker, R. J.; Hoyne, A. L., and Meyer, K. A.: Potassium Deficiency in Bulbar Poliomyelitis, J. A. M. A. **146**:1017, 1951. Gillespie, C. E., and Sanders, R. L.: Use of Potassium Therapy Following Subtotal Gastrectomy, A. M. A. Arch. Surg. **63**:9, 1951. Craig, P.; Zak, B.; Iseri, L. T.; Boyle, A. J., and Myers, G. B.: The Spectrophotometric Determination of Magnesium in Human Serum, Am. J. Clin. Path. **21**:394, 1951. Daum, K.; Tuttle, W. W.; Kisgen, R.; Imig, C. J.; Martin, C.; Klapka, M. R.; Sheehan, J., and Agle, M.: Utilization of Nitrogen, Phosphorus, Calcium, and Iron by Men on Different Breakfasts, J. Am. Dietet. A. **27**:475, 1951. Goldenberg, H., and Sobel, A. E.: Calcification: V. Influence of Fluoride and Cyanide Ions in the Presence and Absence of Magnesium, Proc. Soc. Exper. Biol. & Med. **78**:719, 1951. Taylor, W. H.: Use and Interpretation of the Fantus Estimation of Urinary Chloride, Brit. M. J. **2**:1125, 1951. Myers, W. P. L., and Kirklin, J. W.: Importance of Attention to Potassium Disturbances in Surgical Patients, Proc. Staff Meet., Mayo Clin. **27**:94, 1952. Greisheimer, E. M.: Fluid Therapy, J. Am. M. Women's A. **7**:21 and 54, 1952.

303. Snavely, J. R.: Hepatic Factors in Salt and Water Metabolism, Am. J. M. Sc. **223**:96, 1952.

304. Danowski, T. S.: Newer Concepts of the Role of Sodium in Disease, Am. J. Med. **10**:468, 1951.

305. Forbes, G. B., and Perley, A.: Estimation of Total Body Sodium by Isotopic Dilution: I. Studies on Young Adults, J. Clin. Invest. **30**:558, 1951; II. Studies on Infants and Children, ibid. **30**:566, 1951.

306. Danowski, T. S.; Greenman, L.; Peters, J. H., and Mateer, F. M.: An Introduction to Experimental and Clinical Studies of Carboxylic Cation Exchange Resins, J. Clin. Invest. **30**:979, 1951. Danowski, T. S.; Greenman, L.; Mateer, F. M.; Parsons, W. B.; Weigand, F. A.; Mermelstein, H., and Peters, J. H.: Carboxylic Cation Exchange Resin Effects in Dogs, ibid. **30**:984, 1951. Greenman, L.; Peters, J. H.; Mateer, F. M.; Weigand, F. A.; Wilkins, D.;

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of these substances. It is pointed out that these resins have a relatively low efficiency in deviating sodium to the stools and should not be considered as a substitute for a low-sodium diet. With prolonged use, they may produce acidosis, hyperchloremia, and potassium deficiency. At present their greatest usefulness lies in permitting patients who require low-sodium therapy to ingest more palatable diets than would be possible under a regimen of rigid sodium restriction.

Calcium and Phosphorus.—Harrison and Harrison [307] have studied calcium absorption by means of radioactive Ca^{45} administered as calcium chloride to normal, rachitic, and vitamin D-treated rats. Findings suggested that vitamin D is effective in increasing calcium absorption only under conditions in which the calcium of the intestinal contents is poorly soluble. Steggerda and Mitchell [308] were unable to demonstrate any relation between the amount of butterfat in the diet and the absorption or utilization of calcium. Baylor and associates [309] have studied the effect of calcium administered intravenously upon the excretion of calcium and phosphorus in urine and feces and upon calcium-phosphorus balance. Leichsenring and associates,[310] who investigated calcium and phosphorus metabolism at various levels of intake in a group of college women, concluded that the level of phosphorus in the diet was an important factor in determining calcium utilization. Lovelock and Burch [311] have presented findings which suggest that the element calcium is not essential for blood coagulation. The effectiveness of anticoagulants was found to be more closely related to their total ionic charge than to their efficiency in removing calcium from solution. Lutwak-Mann [312] has studied the distribution of phosphorus in the bone marrow of normal animals, of animals on deficient diets, and of animals exposed to substances known to produce damage to the bone marrow. Edmondson

Tarail, R.; Rhodes, G., and Danowski, T. S.: Biochemical Changes Accompanying the Ingestion of a Carboxylic Cation Exchanger in the Hydrogen, Ammonium, Sodium, Potassium, or Calcium Form, ibid. **30**:995, 1951. Peters, J. H.; Danowski, T. S.; Greenman, L.; Weigand, F. A.; Clarke, C. E.; Gower, K.; Mateer, F. M., and Tarail, R.: Acidifying and Non-Acidifying Carboxylic Resin Mixtures Used Alone and with ACTH or Cortisone, ibid. **30**:1009, 1951. Mateer, F. M.; Erhard, L. H.; Price, M.; Weigand, F. A.; Peters, J. H.; Danowski, T. S.; Tarail, R., and Greenman, L.: Sodium Restriction and Cation Exchange Resin Therapy in Nephrotic Children, ibid. **30**:1018, 1951. Greenman, L.; Peters, J. H.; Mateer, F. M., and Danowski, T. S.: Probable Clinical Utility of Cation Exchange Resins, ibid. **30**:1027, 1951. Danowski, T. S.; Greenman, L.; Peters, J. H.; Mateer, F. M.; Weigand, F. A., and Tarail, R.: The Use of Cation Exchange Resins in Clinical Situations, Ann. Int. Med. **35**:529, 1951.

307. Harrison, H. E., and Harrison, H. C.: Studies with Radiocalcium: The Intestinal Absorption of Calcium, J. Biol. Chem. **188**:83, 1951.

308. Steggerda, F. R., and Mitchell, H. H.: The Calcium Balance of Adult Human Subjects on High and Low Fat (Butter) Diets, J. Nutrition **45**:201, 1951.

309. Baylor, C. H.; Van Alstine, H. E.; Keutmann, E. H., and Bassett, S. H.: The Fate of Intravenously Administered Calcium: Effect on Urinary Calcium and Phosphorus, Fecal Calcium and Calcium-Phosphorus Balance, J. Clin. Invest. **29**:1167, 1950.

310. Leichsenring, J. M.; Norris, L. M.; Lamison, S. A.; Wilson, E. D., and Patton, M. B.: The Effect of Level of Intake on Calcium and Phosphorus Metabolism in College Women, J. Nutrition **45**:407, 1951.

311. Lovelock, J. E., and Burch, B. M.: Blood Clotting: The Inhibition by Anions and the Function of Calcium, Biochem. J. **48**:xxxiv, 1951.

312. Lutwak-Mann, C.: Some Aspects of Phosphorus Metabolism in Bone Marrow, Biochem. J. **49**:300, 1951.

and associates [313] have observed hypocalcemia in 19 of 27 patients with acute pancreatitis. The decrease in serum calcium concentration usually occurred between the second and fifth day of illness and persisted after serum amylase had returned to normal. Determination of concentration of calcium in serum was felt to be of definite aid in the diagnosis of acute pancreatitis, particularly when there was complete destruction of pancreatic tissue or when the patient was seen late in the course of the disease. Mosher and associates [314] have presented a method for the estimation of calcium in plasma by means of the flame spectrophotometer, and King [315] has presented a simple chemical method for calcium determination.

Iron.—New developments in iron metabolism have been reviewed by Johnston.[316] Studies of the rate of disappearance of radioactive iron from the plasma of normal subjects and patients with various erythropoietic disorders have indicated that the turnover rate of Fe^{59} furnishes a reliable index of the rate of erythrocyte formation.[317] It is of considerable interest that the rate of iron uptake from the plasma has been found to be increased in patients with untreated pernicious anemia, suggesting that the red cells and their precursors which are formed in the megaloblastic marrow are broken down *in situ*. After treatment with vitamin B_{12}, the rate of turnover returns to normal. Moore and Dubach [275] have studied the absorption of radioactive iron incorporated into foods and found that in healthy subjects less than 10% is absorbed. The rate of absorption was not influenced by the administration of hydrochloric acid or antacids but was increased by the addition of ascorbic acid to the diet. No evidence of increased efficiency of iron absorption by subjects with iron deficiency was observed, contrary to findings reported previously by other investigators. Hahn and associates [318] have demonstrated a threefold or greater increase in the absorption of iron by pregnant women during the later as compared with the earlier months of gestation. The total red cell mass of the newborn infant contained only 10% as much of the administered iron as did that of the mother. The observations of Lund [319]

313. Edmondson, H. A.; Berne, C. J.; Homann, R. E., Jr., and Westman, M.: Calcium, Phosphorus, Magnesium and Amylase Disturbances in Acute Pancreatitis, Am. J. Med. **12**:34, 1952.

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indicate that the total hemoglobin mass of the pregnant female increases by an average amount of 15%. If iron is administered during pregnancy, this increase may be as great as 24%. These changes are masked by a concomitant rise in plasma volume averaging 48%.

It has been observed by several investigators that the rate of blood regeneration following phlebotomy is dependent chiefly upon the state of body stores of iron.[320] Hemoglobin formation following repeated phlebotomies in subjects with hemochromatosis is far more rapid than would be expected in subjects with normal iron stores.[320b]

Studies of the intravenous administration of saccharated iron oxide have confirmed its effectiveness and rapidity of action in the treatment of iron deficiency anemias.[321] However, toxicity of this compound, when so administered, limits its use to the treatment of those patients with severe iron-deficiency anemia who are unable to tolerate iron administered orally. The dosage should be carefully calculated and due consideration given to the potential dangers attendant upon prolonged administration.[322] Saccharated iron oxide has not been found effective in the treatment of hypoferremia due to infection and was recovered in large amounts from the liver and spleen of two patients with infection who received this medicament and came to autopsy.[323] Further investigation of the apparent deviation of iron from its usual metabolic pathways in the anemias of infection has suggested that the hypoferremia is mediated, at least in part, by the adrenal cortex.[324] Similar hypoferremia has been produced experimentally by a number of agents which cause varying degrees of stress and by the administration of adrenal cortical extracts and corticotropin. Adrenalectomy diminished the rate of disappearance from the plasma of iron administered intravenously, as did the injection of colloidal thorium dioxide, suggesting that the reticuloendothelial system may be stimulated to take up iron by the adrenal cortical hormones.

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A number of reviews and case reports have reemphasized the frequency and severity of poisoning due to accidental ingestion by children of large amounts of ferrous sulfate.[325]

Copper, Cobalt, Beryllium.—Wintrobe [326] has recently reviewed current knowledge of copper metabolism and reported observations on copper deficiency in swine. The concentration of copper in human milk has been found to be relatively constant, regardless of dietary intake, suggesting that some regulatory mechanism may be operative.[327] The copper content of human milk was considerably higher than that of cow's milk, which raises the question whether copper should be considered in planning artificial feeding of infants.

Shen and Homburger [328] have studied anemia in 116 patients with advanced carcinoma and have found that it is usually unrelated to the presence or absence of metastasis to bone. They stated that cobaltous chloride may increase erythropoiesis in such patients, even after the failure of therapy with iron and/or liver extract. The experimental production of macrocytic anemia in animals exposed to beryllium by inhalation has been reported by Stokinzer and Stroud.[329]

MISCELLANEOUS

Quiring [330] reported that West Indian and New Orleans Negroes showed remarkable uniformity in blood pressure, height, and weight, in spite of differences in economic and other conditions. Average basal metabolic rates were within the limits established for a white population. Higgons and associates [331] in a long-term study of 73 children with rheumatic heart disease found a positive correlation between the clinical history and the growth record using the Wetzel Grid. Grid deflections to the right may precede clinical symptoms and be of assistance in the diagnosis of active disease. Eighty-six per cent of the children fell in the median or stocky physique channels.

The nutritive value of school lunches was estimated by chemical analysis and compared with the value determined by calculation from tables of food composition.[332] The average calculated values for thiamine and ascorbic acid were 34 and 57% higher, respectively, than the analytical values, while those for riboflavin were 20%

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lower. Methods for obtaining dietary histories and their relative accuracy have continued to receive attention.[333] Nutrition surveys have been conducted in school-children and in young women, and dietary and biochemical data are presented.[334] Problems and methods of assessment of nutritional status have been considered in a recent report of the joint Food and Agriculture Organization—World Health Organization Expert Committee on Nutrition.[335] The reliability of clinical signs in such assessment has been discussed by Bransby and Hammond.[336] Problems relative to feeding of the aged have been considered by Walker [337] and Donahue.[338]

The role of labile methyl groups in intermediary metabolism has been considered in a recent editorial.[339] A study of absorption of various nutrients from the intestinal tract using portal anastomotic veins was reported by Bean and collaborators.[340] The concentration and distribution of adenine nucleotides in the blood were investigated by Albaum and associates.[341] Toaff [342] has observed cytologic changes in the oral epithelium in nutritional deficiency with the use of the smear technique. The study by Ludovici and Axelrod [343] of circulating antibodies in vitamin-deficiency states in rats may have implications in human nutrition. Severe impairment of antibody response was noted in pantothenic acid, pyridoxine, and pteroylglutamic acid deficiency, moderate impairment in riboflavin, thiamine, biotin, vitamin A, and nicotinic acid-tryptophane deficiency, and no impairment in vitamin B_{12} and vitamin D deficiency.

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Several clinical studies of malnutrition in prisoners of war have been reported.[344] A discussion of nutritional factors in heart disease and of the influence of these factors on the management of the cardiac patient was presented by Durant.[345] A pamphlet on therapeutic nutrition, with special reference to military situations, was published by a committee of the Food and Nutrition Board of the National Research Council.[346] The therapeutic indications for vitamins in mixtures was discussed by Spies and associates.[347] The importance of cereal enrichment as a public health measure has been emphasized in several recent publications.[348] Problems relative to chemical additives, pesticides, and adulterants in food have received much attention.[349] An excellent summary of general principles underlying the use of chemical additives in food has been prepared by the Food Protection Committee of the National Research Council.[350]

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Case Reports

DIPHTHEROID SUBACUTE BACTERIAL ENDOCARDITIS SUCCESSFULLY TREATED WITH BACITRACIN

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THE ADVENT of antibiotic therapy has brought about a dramatic improvement in the recovery rate from subacute bacterial endocarditis. At present, according to reports in the literature, the outlook for cure in patients treated with adequate penicillin schedules varies from 80 to 95%. These reports contrast sharply with a spontaneous recovery rate of 1% and the recovery rate of 5% attributable to sulfonamide therapy reported in the series collected by Lichtman.[1] Friedberg,[2] however, pointed out that the actual clinical recovery rate in his series was only about 70%, which was much lower than the bacteriological cure rates being reported. He attributed this difference in results to a variety of reasons, the most important of which were failure to isolate the organism, improper diagnosis with consequent delay in proper treatment, and inadequate treatment schedules. The most favorable results, according to Bloomfield,[3] are obtained in fairly young persons with minimal valve damage or relatively mild congenital defects, in whom an early diagnosis can be made and the organism is found to be highly sensitive to penicillin.

In a significant group of cases bacterial endocarditis is caused by unusual organisms or unresponsive strains against which penicillin therapy proves ineffective. This has led to the use of some of the newer antibiotics in combination with penicillin in attempts to eradicate the infection. In some instances as many as three or more antibiotics have been used together, with favorable results, after treatment with penicillin alone has been futile.

The newest addition to this armamentarium is bacitracin. Although the first clinical reports on this antibiotic appeared in 1945,[4] it is only recently that preparations considered safe for prolonged parenteral use have been introduced. The first

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2. Friedberg, C. K.: Subacute Bacterial Endocarditis: Revision of Diagnostic Criteria and Therapy, J. A. M. A. **144**:527, 1950.

3. Bloomfield, A. L.: The Present Status of Treatment of Subacute Bacterial Endocarditis, Circulation **2**:801, 1950.

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large series of cases was reported by Meleney and his group in December, 1949.[5] These authors published the results obtained in 270 patients treated systemically with bacitracin for a variety of surgical and medical conditions. Since then, an increasing number of cases of successful parenteral treatment with bacitracin have been reported.

Friedberg and Bader[6] described a case of Staphylococcus endocarditis which failed to respond to massive doses of penicillin, chloramphenicol (chloromycetin®), and aureomycin, with cure when bacitracin was given in conjunction with penicillin and aureomycin. There have been other recent reports of the use of bacitracin together with penicillin in the successful treatment of subacute bacterial endocarditis previously resistant to penicillin alone or in combination with other antibiotics.[7]

We have not been able to find any prevous report of subacute bacterial endocarditis treated with bacitracin alone. In the case here presented of subacute bacterial endocarditis caused by a diphtheroid bacillus, the patient had been treated unsuccessfully with very large doses of penicillin, first alone and then in combination with other antibiotics. Later laboratory studies revealed marked sensitivity of the organism to bacitracin. Rapid sterilization of the blood stream with concomitant clinical improvement resulted when the patient was given bacitracin alone. After discontinuance of therapy, the patient has remained well, has gained weight, and has had no elevation of temperature above normal. Repeated blood cultures taken at frequent intervals have all been sterile. At the time this report is written the patient appears cured.

REPORT OF CASE

F. N., a 49-year-old white man (MCH 9538), was admitted to the hospital Sept. 4, 1951, and discharged Feb. 27, 1952.

This first admission of a 49-year-old white male stock clerk to Morrisania City Hospital was because of the chief complaint of severe and progressive shortness of breath of two weeks' duration. He gave a history of exertional dyspnea first noticed two years prior to admission, but this had never been severe enough to incapacitate him until the onset of his present illness. The patient had no chills and was unaware of fever.

The past history included the following pertinent facts: Ten years prior to admission he had been rejected by the Army because of a heart murmur; this was the first he was aware of any such condition. The presence of this murmur was confirmed subsequently by two private physicians. There was no history suggestive of previous rheumatic fever or of any other serious illness. There had been no upper respiratory infection or surgical procedures involving the nose, throat, or teeth preceding the onset of symptoms.

Physical examination revealed a pale, well-developed and well-nourished white man who did not appear acutely ill. There was no icterus or cyanosis. No petechiae were noted. The pupils were equal and reacted well to light and in accommodation. The extraocular muscles were intact. Examination of the fundus showed no abnormality. The lungs were clear to percussion and auscultation. The blood pressure was 120/60. The heart was enlarged to the left. The rhythm

5. Meleney, F. L.; Longacre, A. B.; Altemeier, W. A.; Reisner, E. H., Jr.; Pulaski, E. J., and Zintel, H. A.: The Efficacy and Safety of Intramuscular Administration of Bacitracin in Various Types of Surgical and Medical Infections, with an Analysis of 270 Cases, Surg., Gynec. & Obst. **89**:657, 1949.

6. Friedberg, C. K., and Bader, M. E.: Acute Staphylococcic Endocarditis Cured with the Aid of Bacitracin, J. A. M. A. **147**:46, 1951.

7. (*a*) Volini, I. F., and Kadison, E. R.: Simultaneous Bacitracin and Penicillin Therapy in Subacute Bacterial Endocarditis: A Report on 3 Cases, Am. Pract. & Digest Treat. **2**:13, 1951. (*b*) Wallach, R., and Pomerantz, N.: Combined Antibiotic Therapy, A. M. A. Arch. Int. Med. **88**:840, 1951.

was completely irregular. There was a pulse deficit, with a ventricular rate of 150 and a pulse rate of 125. Systolic and diastolic murmurs were heard at the apex. The abdomen was soft and nontender. A smooth liver edge was felt 4 fingerbreadths below the right costal margin. There was a palpable spleen. The ankles showed 2+ bilateral edema. Results of neurological and rectal examinations were normal. Circulation tests revealed an arm-to-lung (ether) time of 19 seconds and arm-to-tongue (dehydrocholic acid [decholin®]) time of 30 seconds. Venous pressure was 270 mm. of water. The red blood cell count was 4,600,000, and the hemoglobin concentration was 12.5 gm. per 100 cc.

A diagnosis of rheumatic heart disease in congestive heart failure with possible subacute bacterial endocarditis was made, and blood was taken for cultures. Treatment for cardiac failure was instituted, including digitalization and administration of mercurial diuretics, low-sodium diet, and sedatives. Soon after his admission persistent temperature elevation became manifest. Treatment with penicillin was begun on Sept. 8, 1951, with 10,000,000 units given daily. No response was obtained. Two grams of streptomycin daily was added on Sept. 18 and 2 gm. of terramycin daily on the next day. On Sept. 20, the penicillin dose was increased to 20,000,000 units. On Sept. 24, the dose of terramycin was increased to 4 gm. per day, and use of streptomycin was discontinued. The patient's condition did not improve. Penicillin administration was discontinued on Sept. 26, and that of terramycin on Oct. 14.

On Oct. 22, a culture of blood which had been taken on Oct. 8 was reported by the laboratory as showing growth of a diphtheroid bacillus. This was the first positive blood culture reported, though many had been made. Additional cultures were reported positive for the same organism on Oct. 24 and 25. Sensitivity tests were done and showed the organism to be sensitive to both penicillin and terramycin. Accordingly, therapy with 16,000,000 units of penicillin and 4 gm. of terramycin daily was begun again. The clinical picture did not improve, and the patient continued to have a temperature elevation for the next two weeks. At this point it was deemed advisable to discontinue all therapy, so that fresh cultures could be made and new sensitivity tests performed uninfluenced by antibiotics.

Blood cultures made on Dec. 3, 15, and 19 were again reported as positive for a diphtheroid. These cultures were done with great care to avoid contamination. Sensitivity tests on the new cultures showed the organism to be highly sensitive to bacitracin, moderately sensitive to aureomycin and terramycin, slightly sensitive to streptomycin, and resistant to penicillin, chloramphenicol, and polymyxin. In view of the marked sensitivity of the organism to bacitracin and the failures with the antibiotics previously used, it was decided to try a course of therapy with bacitracin alone. On Jan. 10, 1952, the administration of 60,000 units of bacitracin intramuscularly daily in divided doses of 20,000 units every eight hours was begun. The injections were given alternately in each buttock. Ten grains (0.65 gm.) of sodium bicarbonate was administered orally with each dose, and the patient was given liberal amounts of fluids. In order to minimize any local effects of the injections, the material was prepared by dilution in isotonic sodium chloride solution containing 2% procaine hydrochloride. Therapy was maintained on an uninterrupted schedule for 31 days, for a total of 1,860,000 units of bacitracin.

The patient tolerated the drug very well. The only untoward reaction observed was a daily temperature elevation to levels higher than those prior to institution of treatment. This began on the second day of therapy and continued for five days. The temperature then dropped to normal levels and remained there throughout the rest of the course of treatment. Although careful search was made, no apparent cause for this temperature rise could be found, and it was thought possibly a reaction to the bacitracin. Abnormal urinary findings, which were noted soon after the onset of therapy, persisted during only the first two weeks of treatment and then disappeared, although no change in the treatment schedule was made. Signs of nephrotoxicity included albuminuria, the reactions varying from trace to 2+, coarse and fine granular casts, and occasional red blood cells. After the completion of therapy, microscopic urine examinations showed a completely normal condition, and there was no albuminuria. The blood urea nitrogen determined at weekly intervals never rose above normal levels. A phenolsulfonphthalein test performed after therapy with bacitracin showed 65% excretion in two hours, and this result compared favorably with the 55% excretion noted prior to treatment.

After 14 days of parenteral bacitracin therapy, the patient's temperature reached normal levels and remained there for the rest of his hospital stay. Numerous blood cultures were reported

as showing no growth. The patient began to look and feel better, and his appetite improved. At the time of discharge from the hospital, on Feb. 27, 1952, the only abnormal physical findings were the systolic and diastolic apical murmurs, slow auricular fibrillation, and enlargement of the spleen to 2 fingerbreadths below the left costal margin. The patient had no complaints, had regained some weight, and was eager to go home.

Since his discharge, he has been followed closely in the clinic, where repeated blood cultures have all been negative. Rectal temperatures recorded three times a day by the patient have never exceeded 99.4 F. He appears to be clinically cured.

COMMENT

The causative organism in this case is of particular interest, since subacute bacterial endocarditis due to a diphtheroid is so rare an occurrence. For many years the pathogenicity of diphtheroids has been widely debated, and any claim for the indictment of this organism as the etiological agent in a case of subacute bacterial endocardtis must be made with great caution. Libman and Friedberg [8] found that in 90% of cases subacute bacterial endocarditis was caused by the nonhemolytic Streptococcus, usually of the viridans group, and that among the remaining 10% could be found a wide variety of causative organisms, including diphtheroids. They caution that care must be exercised when organisms having the morphology of diphtheroids are observed in stained spreads of vegetations, because they may really represent a diphtheroid phase of nonhemolytic streptococci. The same care must be exercised when organisms of diphtheroid appearance are recovered in blood cultures. In 1931 Jensen and Morton [9] drew special attention to this subject, and they described two strains of organisms which in blood agar cultures appeared as diphtheroids, but which were found to be nonhemolytic streptococci when cultured in dextrose brain broth.

The exact nature of the diphtheroids as a group is not too well agreed upon, but most bacteriologists feel that the term includes a wide variety of organisms which look like the diphtheria bacillus. According to Park and Williams,[10] the diphtheroids include a large number of small, more or less irregular bacilli bearing some morphological resemblance to the diphtheria bacillus. They are common inhabitants of the nasopharynx. They differ from Corynebacterium diphtheriae by not producing an extracellular toxin and by different fermentative reactions on carbohydrates. They are considered to be nonpathogens as a rule.

Lamana [11] reviewed the role of diphtheroid-like streptococci as causative agents of endocarditis in 1944. Citing various authors, he collected 11 cases of endocarditis caused by streptococci with diphtheroid appearance. Most were cases of subacute bacterial endocarditis, but one reported by Tow and Wechsler [12] was described as

8. Libman, E., and Friedberg, C. K.: Subacute Bacterial Endocarditis, New York, Oxford University Press, 1941.

9. Jensen, L. B., and Morton, H. B.: The Diphtheroid Phase of Streptococci, J. Infect. Dis. **44**:425, 1931.

10. Park, W. H., and Williams, A. W.: Pathogenic Microörganisms, Philadelphia, Lea & Febiger, 1939, p. 442.

11. Lamana, C.: A Non-Life Cycle Explanation of the Diphtheroid Streptococcus from Endocarditis, J. Bact. **47**:327, 1944.

12. Tow, A., and Wechsler, H. F.: Diphtheroid Bacillus as a Cause of Acute Endocarditis, Am. J. Dis. Child. **44**:156, 1932.

an acute vegetative endocarditis of the mitral valve manifesting widespread embolic phenomena. In this case a diphtheroid bacillus was recovered from the blood during life and on autopsy, but smears of the vegetations showed a Streptococcus as well. In none of these cases was the organism ever substantiated to be anything more than a pleomorphic form of Streptococcus. Wallach and Pomerantz [7b] described a case of subacute bacterial endocarditis first seen in 1948, when a viridans Streptococcus was cultured from the blood stream. When the patient had a recurrence in 1949, an organism having the appearance of a diphtheroid was recovered. Review of the more recent literature has not disclosed another case of subacute bacterial endocarditis in which diphtheroids were proved to be the causative organism.

With these facts in mind, we have attempted to establish definite laboratory proof of the exact nature of the organism cultured from the blood stream of our patient. The following is a brief summary of the procedures carried out:

The media and technique routinely used at Morrisania City Hospital are as follows: One bottle of thioglycollate broth and one bottle of soy trypticase broth, each containing 50 cc. of medium, and a plain agar pour plate are used. Approximately 12 cc. of blood is drawn. Five cubic centimeters is put into each bottle and 2 cc. into the plate. The blood cultures are incubated at 37 C., under increased carbon dioxide tension if indicated, and subcultured at regular intervals.

In this case the micro-organisms recovered from all the blood cultures were consistent in colony formation, in microscopic morphology, and in the length of time necessary for appearance in the cultures. The diphtheroid became apparent on Gram-stained smear on the eighth day of incubation. In order to rule out the possibility of its being a contaminant, after the organism was recovered a second time, daily gross examinations of the bottles and plates were made, but subcultures and smears were not done until the eighth day. With this procedure, the same organism was recovered from four more blood cultures.

The colony characteristics on blood agar plate were typical and distinct, always following the same pattern. In 24 hours, at 37 C. incubation, there was a dry faint film, which in 48 hours exhibited larger round smooth white tenacious colonies. As the colonies aged, they developed some yellow pigment and even greater tenacity. There was no change in the color of the blood plate.

The Gram-stained smear showed the typical morphology of a diphtheroid, Gram positive with some Gram-negative forms and palisade arrangements, sometimes quite slender when first isolated but becoming club-shaped and irregular in morphology in subcultures. The methylene blue stain of the organisms grown in the broth, blood agar, and Loeffler's medium showed mostly club-shaped forms with metachromatic granules but without typical C. diphtheriae morphology.

The organism was nonmotile. The indole reaction was negative. Litmus milk remained unchanged. There was acid without gas formation in dextrose. There was no reaction in maltose, mannitol, lactose, and sucrose. It grew equally well in atmospheric oxygen and carbon dioxide at 37 C. It grew poorly at room temperature and under anaerobic conditions (nitrogen-replacement method). Subcultures grew well on the surface of the thioglycollate broth, forming small granules scarcely visible in 24 hours and becoming increasingly larger within the next two or three days. It grew well on chocolate agar, human blood, and plain agar; it did not grow on MacConkey's agar.

To rule out the possibility the organism might be a pleomorphic Streptococcus, the following tests were conducted:

1. Daily subcultures were made for two weeks using three media, human blood agar, soy trypticase broth, and thioglycollate broth. Smears were made daily and stained with Gram stain. Subsequently subcultures were made every week and now monthly. Up to now there has been no evidence of a coccoid form; the morphology has been consistently bacillary with typical diphtheroid appearance. At no time has there been any evidence of true branching.

2. Two mice were inoculated intraperitoneally with 1 cc. of a 24-hour saline suspension from a blood agar plate. Peritoneal fluid withdrawn from the mice 24 hours later was sterile. The mice showed no reaction, and there were no abnormal findings when they were killed after two weeks.

3. The hydrogen peroxide test for detecting the presence of catalase gave a positive result.

4. This organism did not grow well anaerobically, either in the Brewer jar containing nitrogen gas or in the lower anaerobic portion of the thioglycollate tube. Streptococci usually grow abundantly under such anaerobic conditions.

In view of the laboratory evidence, we feel justified in assuming that the organism causing the subacute bacterial endocarditis in this patient was a true diphtheroid and not a pleomorphic Streptococcus, as seems to have been the case in most instances previously reported.

We have not been able to find in the literature another case of endocarditis treated with bacitracin alone. In addition to the case of Friedberg and Bader [6] already cited, Volini and Kadison [7a] have reported three cases of subacute bacterial endocarditis due to streptococci refractory to penicillin alone with cure when bacitracin was given as well. In the case of Wallach and Pomerantz,[7b] with failure of response to large doses of other antibiotics, cure was also obtained by the addition of bacitracin to the penicillin therapy.

In this case we administered bacitracin in the amount of 60,000 units daily for 31 consecutive days for a total of 1,860,000 units. Meleney [13] has given similar and even larger amounts over longer periods without any serious reactions. He feels that with use of the less toxic lots now being manufactured, if the dose is limited to a maximum of 80,000 to 100,000 units daily, evidences of kidney damage will be minimal and always reversible. This was borne out in our case, since the early and minimal signs of nephrotoxicity subsided by the end of the second week of treatment, and tests of renal function at the completion of therapy showed no residual effects.

The rise in temperature experienced by our patient from the second to eighth days may have been a side-effect of parenteral bacitracin therapy, since no other explanation for the temperature elevation above its former levels could be found. A febrile reaction to parenterally administered bacitracin has not to our knowledge been previously reported.

13. Meleney, F. L.; Johnson, B. A., and Teng, P.: Further Experiences with Local and Systemic Bacitracin in the Treatment of Various Surgical and Neurosurgical Infections and Certain Related Medical Infections, Surg., Gynec. & Obst. **94**:401, 1952.

SUMMARY

In a case of subacute bacterial endocarditis due to a diphtheroid bacillus, treatment with bacitracin alone was successful after previous therapy with large amounts of other antibiotics singly and in combination had failed.

Bacteriological investigations were carried out to establish the causative organism as a true diphtheroid.

A total dose of 1,860,000 units of bacitracin was given parenterally over a 31-day period without evidence of any serious or permanent toxic effects.

A review of the literature has not disclosed another case of cure of subacute bacterial endocarditis with use of bacitracin alone.

Elevation of temperature was noted during the first week of bacitracin therapy; this may have been a side-effect of its parenteral administration.

The bacitracin used in this case was supplied through the courtesy of Dr. Frank L. Meleney.

Miss Dorathea Richtberg and Miss Mary Spirito, of the Bacteriology Laboratory of Morrisania City Hospital, assisted in the isolation and identification of the causative organism.

ASYMPTOMATIC ARTERIOVENOUS FISTULA OF THE LUNG

Report of a Case with Surgical Cure

THEODORE J. TALBOT, M.D.
AND
JACOB J. SILVERMAN, M.D.
STATEN ISLAND, N. Y.

THE RECOGNITION of pulmonary arteriovenous fistula, once thought to be extremely rare, is now reported with increasing frequency. To date there have been at least 53 cases diagnosed, and in 33 of these operation has been performed.[1] Since a pulmonary arteriovenous fistula is amenable to surgical cure, particularly if uncomplicated, the establishment of an early and correct diagnosis becomes important. A group of signs and symptoms has been built around the diagnosis of a pulmonary arteriovenous fistula. The clinician is alerted to this diagnosis whenever a patient, in the absence of congenital heart disease, presents himself with cyanosis, clubbing of the fingers and toes, and polycythemia. It is not sufficiently appreciated, however, that an arteriovenous fistula of the lung can present, at times, an entirely asymptomatic clinical picture. It may masquerade, for example, as a benign-appearing shadow in the pulmonary parenchyma detected only by chance roentgenograms. It is the purpose of this report to present an instance of a completely asymptomatic pulmonary arteriovenous fistula successfully cured by operation. The clue which eventually led to the diagnosis was accidentally found during a routine preemployment roentgenographic examination of the chest. An isolated noncharacteristic pulmonary nodule was discovered. Laminagraphic studies subsequently established the correct diagnosis.

REPORT OF CASE

The patient was a 48-year-old white Jewish man employed as an electrician at a Veterans Administration Hospital. All employees of the hospital are subjected periodically to roentgenographic examinations of the chest. It was in the process of one of these surveys that a small, round shadow in the left lung field above the diaphragm was discovered. He was entirely asymptomatic, but since the nature of the shadow was not clear he was advised to consult his family physician, who in turn referred him to one of us (T. J. T.).

The family history was noncontributory. The patient was married. His wife and two children were always considered healthy. Recent roentgenograms of the chest of the two children were available, and the findings were interpreted as normal. The patient's father died

From the medical service of the Staten Island Hospital.

1. Blades, B., and Beattie, E.: Pulmonary Arteriovenous Aneurysm, J. Mt. Sinai Hosp. **18**:195, 1951.

of arteriosclerotic heart disease at the age of 70, and his mother died after an operation for goiter at the age of 40. An older sister was living and well. There was no family history of cancer, tuberculosis, or unusual bleeding.

The patient's past health was always considered good. There was no history of trauma, pneumonia, or pleurisy. He smoked an average of one package of cigarettes a day. He denied cough, expectoration, dyspnea, chest pain, hemoptysis, weight loss, or anorexia. He did admit,

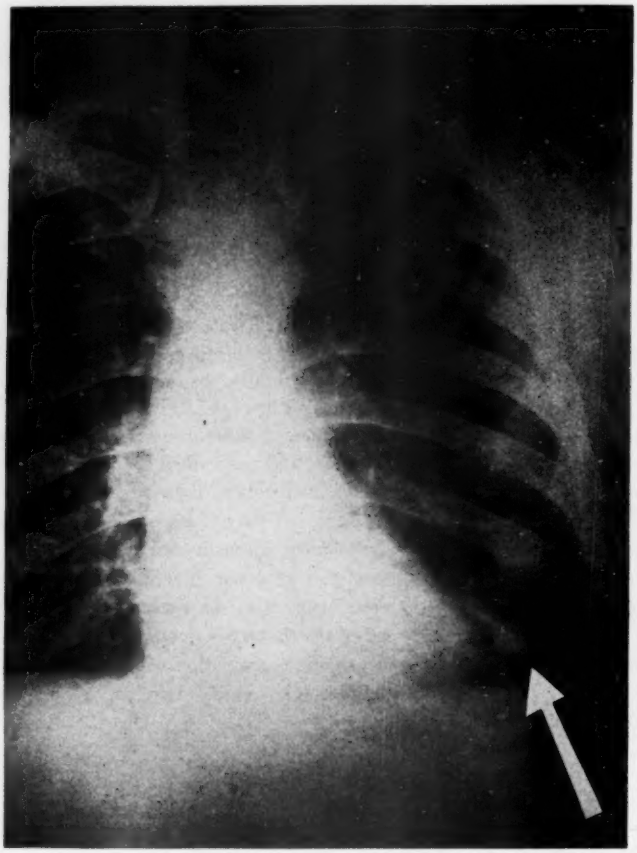


Fig. 1.—Roentgenogram of chest, demonstrating circumscribed area of density just outside apex of heart.

however, on close questioning, that for the past several years he was subject to an occasional inconsequential nosebleed and, at times, momentary dizziness.

On physical examination the patient's nutrition and development were excellent. He was symptom free and afebrile. His blood pressure in each arm measured 135 mm. of mercury systolic and 80 mm. diastolic. The heart sounds were of normal quality and regular; no murmurs were heard. The pulse rate averaged 80 per minute. Auscultation of the lungs revealed normal breath sounds throughout; no rales, adventitious sounds, or extracardiac bruit were detected. There was no cyanosis of the extremities or mucous membranes. Clubbing of

the fingers and toes was absent. The skin was carefully examined for rashes, blemishes, or telangiectasia, and none was found. Results of funduscopic examination of the eyes were not remarkable. The liver and spleen were not palpable. The pulsations in the neck were normal. The red blood cell count was 4,800,000 per cubic millimeter, and the hemoglobin concentration was 14.5 gm. per 100 cc. The white blood cell count was 7,800 per cubic millimeter, with a normal differential count. Results of a urinalysis were normal. The blood Kahn serologic test for syphilis gave a negative reaction. The reaction to a tuberculin test (Mantoux, 1 : 1,000) was positive.

The conventional posteroanterior roentgenogram of the chest (Fig. 1) disclosed a round, circumscribed nodular density measuring 3 cm. in diameter situated above the left hemidiaphragm, adjacent to the cardiac apex. A lateral roentgenographic study contributed no additional information, since on this view the lesion appeared to merge with the cardiac shadow. A

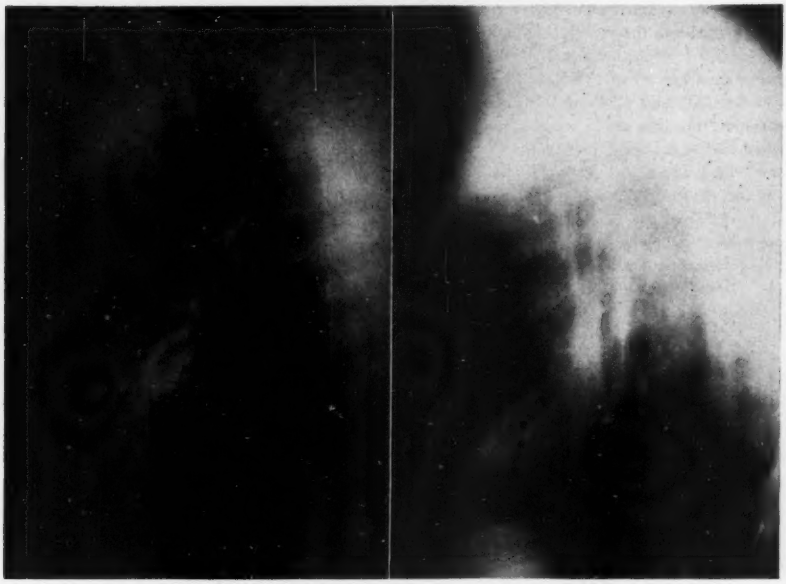


Fig. 2.—Lateral tomograms, demonstrating pulmonary arteriovenous fistula from which two major vessels are shown extending toward hilar region.

posteroanterior tomographic study was likewise disappointing and failed to reveal the lesion, which on fluoroscopic examination seemed to be located anteriorly. During the fluoroscopic study no evidence of pulsation in the area of density or changes in the size of the mass during the Valsalva experiment were detected. Lateral laminagraphic studies, however, were revealing and diagnostic. One such study (Fig. 2) disclosed an area of opacity in the lingula of the left upper lobe and, in addition, outlined two band-like densities extending medially and posteriorly from the round nodular opacity toward the hilar shadow. The central mass appeared well defined and seemed to merge with a large wedge-shaped density subjacently and laterally.

The patient was seen in consultation with Dr. J. Maxwell Chamberlain, thoracic surgeon. An asymptomatic arteriovenous pulmonary fistula was considered a likely possibility. Tuberculoma and neoplasm, however, were also considered in the differential diagnosis.

Since a malignant growth could not be ruled out, an exploratory thoracotomy, without completion of further laboratory investigations, was recommended.

Accordingly, on May 22, 1950, Dr. Chamberlain explored the chest through the seventh left intercostal space. A few cobweb-like adhesions were found between the lingula and the lower lobe, and these were easily divided. On palpation of the lower lobe and upper lobe, no tumor mass could be felt, but in the superior portion of the lingula a pulsating cyst-like mass was palpated, and a diagnosis of arteriovenous fistula was immediately established. These pulsations were visible, as well as palpable. The lesion seemed to be confined to the medial segment of the lingula. The lingular artery was then identified and ligated. After this procedure, pulsations of the arteriovenous fistula quickly ceased. The lingular veins were also identified, and all important vessels leading to and from the bronchus were eventually divided. A segmental resection of the lingula containing the fistula was accomplished without difficulty. The patient reacted well to the operation and made an uneventful recovery.

Pathologic Report (Dr. Margaret Bevans).—The specimen (Fig. 3) was the lingula of the left lung. A greatly dilated vein coursed beneath the pleura on the posterior surface. When the lung was inflated and the vein and artery injected with contrasting colored latex, the blue dye injected into the venous circulation quickly appeared on the surface which had been stripped of pleura. It could be traced by dissection of the large subpleural vein to a tortuous bilobulate aneurysmal sac which was brightly colored with the red dye of the arterial injection medium admixed with blue of the venous circulation. Within the vein for several centimeters beyond the aneurysm the two colors were admixed, and the vein was a deep purple. The aneurysmal sac when filled was approximately 3 cm. in diameter. In addition to the artery that parallels the large vein, there was a second large artery of similar caliber which also entered the aneurysmal sac and ran centrally through the length of the specimen. This branched from the common artery at the hilus of the specimen. Iodopyracet (diodrast®) injection of the circulation revealed a roentgenologic picture similar to that described above. No sections were taken. The diagnosis was arteriovenous aneurysm involving the lingular vessels of the left lung.

COMMENT

In the diagnosis of arteriovenous fistula a great deal of stress has been placed on a group of characteristic signs and symptoms. The diagnostic triad of cyanosis, clubbing of the fingers and toes, and polycythemia has been particularly emphasized. Attention has also been directed to the hereditary nature of this disorder, since in many cases it is associated with multiple hemangiomata of the skin and mucous membranes and a history of hereditary epistaxis is frequently obtained. Depending on the degree of oxygen saturation, a multitude of signs and symptoms resulting from anoxemia may predominate. It has been estimated that cyanosis does not appear until at least 25% of the blood is shunted from the pulmonary artery to the vein.[2] The heart is normal, contrary to the findings in peripheral arteriovenous fistula, where cardiac enlargement is the rule. Occasionally, in patients with pulmonary arteriovenous fistula, the erroneous diagnosis of congenital heart disease is made. A continuous extracardiac murmur, with accentuation of both systolic and diastolic phases at the height of deep inspiration, is diagnostic of an arteriovenous fistula.[3] The angiocardiographic picture, of course, is diagnostic. The polycythemia, which at times is profound, may give rise to a chain of symptoms, particularly those of the central nervous system, such as vertigo, headache, convulsions, and paresis. It is now becoming evident, however, that not all patients with arteriovenous fistula

2. Maklor, P. T., and Zion, D.: Multiple Pulmonary Hemangiomata, Am. J. M. Sc. **211**:261, 1946. Behrend, A., and Baer, S.: Pulmonary Arteriovenous Fistula, S. Clin. North America **30**:587, 1950.

3. Marchand, E. J.; Hejtmancik, M. R., and Herrmann, G. R.: Extracardiac Arteriovenous Fistulas in the Thorax, Am. Heart J. **42**:682, 1951.

Fig. 3.—Specimen of lingula of left lung removed at operation, demonstrating aneurysmal sac of arteriovenous fistula, together with large vessels associated with it. Vein and artery were injected with blue and red contrasting colored latex, respectively. Aneurysmal sac is brightly colored purple from mixture of red dye of arterial injection with blue dye of venous circulation. (The expense of this reproduction was partially defrayed by a contribution of the J. Beeber Co. Inc., New York City, and of the F. Mattern Manufacturing Co., Chicago, to the Research Fund of the Medical Service of the Staten Island Hospital, New York.)

present characteristic signs and symptoms. In fact, symptoms and signs may be entirely absent.[4] It is noteworthy that the arteriovenous fistula in our patient, who was strikingly free of symptoms, was accidentally discovered during a routine roentgenographic survey of the chest. A table comparing the average symptoms and signs in the reported cases of arteriovenous fistula with the findings in our case is presented.

The symptoms and signs in pulmonary arteriovenous fistula would seem to depend on the size and duration of the arteriovenous shunt, since blood entering the fistula fails to reach the capillary bed and, therefore, remains unoxygenated.[5] This relationship, however, is not always demonstrated. It is difficult to understand, for example, why our patient, who showed an arteriovenous fistula of considerable magnitude (Fig. 3), was symptom free. It should be pointed out that the lung has a dual blood supply derived from both the pulmonary and the bronchial vessels. It might be suspected, therefore, that tributaries of both systems may be

Diagnostic Signs and Symptoms of Pulmonary Arteriovenous Fistula Found in the Average Reported Cases Compared with Those in the Present Case

	Reported Cases	Present Case
Cyanosis	Present	Absent
Clubbing	Present	Absent
Polycythemia	Present	Absent
Hemangioma elsewhere (telangiectasia)	Present	Absent
Hemorrhages (nasal, pulmonary, renal)	Present	Occasional nose bleed
Cardiac symptoms: dyspnea, chest pain, weakness	Present	Absent
Hereditary	Present	Absent
Age (young adult)	Present	48 yr.
Extracardiac murmur	Present	Absent
Abnormal shadow in roentgenogram of chest	Present	Present
Pulsating shadow	Present	Absent
Variation in mass size with Valsalva experiment	Present	Absent
Angiocardiographic evidence	Present	Not done
Tomographic evidence	Usually not reported	Present

involved to a varying degree in cases of cavernous hemangioma of the lung, and that in the absence of cyanosis the collateral circulation of the bronchial vascular system plays a more prominent role.[6] With this thought in mind our specimen was carefully examined by Dr. A. A. Liebow, of Yale Medical School. From an anatomical standpoint, the pulmonary arteries have been considered end-arteries with communications existing only among finer capillary networks, whereas the bronchial vessels are arranged in an anastomosing network.[7] However, on the basis of Dr.

4. (*a*) Lindskog, G. E.; Liebow, A.; Kausel, H., and Janzen, A.: Pulmonary Arteriovenous Aneurysm, Ann. Surg. **132**:591, 1950. (*b*) Lawrence, E. A., and Rumel, W. R.: Arteriovenous Fistula of the Lung, J. Thoracic Surg. **20**:142, 1950. (*c*) Carswell, J., Jr.: Arteriovenous Fistula of the Lung, with Report of Case, ibid. **19**:789, 1950.

5. Moyer, J. H., and Ackerman, A. J.: Hereditary Hemorrhagic Telangiectases Associated with Pulmonary Arteriovenous Fistula in 2 Members of a Family, Ann. Int. Med. **29**:775, 1948.

6. Lawrence and Rumel.[4b] Maier, H. C.; Himmelstein, A.; Riley, R. L., and Bunim, J.: Arteriovenous Fistula of the Lung, J. Thoracic Surg. **17**:13, 1948. Liebow, A. A.; Hales, M. R., and Lindskog, G. E.: Enlargement of Bronchial Arteries, and Their Anastomoses with Pulmonary Arteries in Bronchiectasis, Am. J. Path. **25**:211, 1949.

7. Miller, W. S.: The Lung, Springfield, Ill., Charles C Thomas, Publisher, 1947.

Liebow's examination it was impossible to assign sufficient collateral circulation to the bronchial artery to explain the absence of any signs or symptoms.

The course of pulmonary arteriovenous fistula is notoriously unpredictable. It is for this reason that the diagnosis of pulmonary arteriovenous fistula should be established early and surgical treatment given. Instances have been reported in the newborn, but diagnosis usually is not established until after puberty. Arteriovenous fistulae enlarge at varying rate in different persons. Fatalities may occur at any time from uncontrollable pulmonary hemorrhage. Thrombosis and subacute bacterial endocarditis at the site of the fistula are also serious threats to the life of the patient.

Roentgenologic studies are of supreme importance in the diagnosis of pulmonary arteriovenous fistula.[8] We wish to point out that the value of tomographic studies has not been stressed sufficiently.[9] The tomograms in our case were of sufficient diagnostic value to enable one to make a correct preoperative diagnosis. In the diagnosis of arteriovenous fistula angiocardiographic studies are usually recommended, especially for detecting multiple lesions. The findings by this technique are pathognomonic when there is prompt filling of the aneurysm with the dye and delineation of afferent and efferent vessels. Angiocardiographic studies, however, are not generally available, and deaths during this procedure have been reported. We believe that the laminagraphic studies demonstrated in our patient on the lateral projectional views (Fig. 2) were as diagnostic as angiocardiograms would have been.

SUMMARY

An arteriovenous fistula of the lung, successfully removed by surgery, is reported in an asymptomatic 48-year-old man.

In the differential diagnosis of a nodule in the lung, an arteriovenous fistula should be considered. An arteriovenous fistula may present itself solely as an isolated nodule in the lung parenchyma.

Laminagraphic studies are recommended whenever a pulmonary arteriovenous fistula is suspected. Abnormal vessels leading to and from the aneurysm may be identified by this technique.

The symptoms and signs of pulmonary arteriovenous fistula vary widely. The absence of the diagnostic triad of polycythemia, clubbing of the fingers and toes, and cyanosis, as well as of other signs, in arteriovenous fistula is discussed.

8. Lindgren, E.: Roentgen Diagnosis of Arteriovenous Aneurysm of the Lung, Acta radiol. **27**:585, 1946.

9. Jones, J. C., and Thompson, W. P.: Arteriovenous Fistula of the Lung: Report of Case of Patient Cured by Pneumonectomy, J. Thoracic Surg. **13**:357, 1944.

News and Comment

ANNOUNCEMENTS

Meeting of American Psychosomatic Society.—The American Psychosomatic Society will hold its tenth annual meeting at Chalfonte-Haddon Hall, in Atlantic City, on Saturday and Sunday, May 2 and 3, 1953.

Titles and abstracts of papers for consideration for the program should be received by the program committee by Dec. 1, 1952. The time allotted for the reading of each paper will be 20 minutes. The committee is interested in investigations in the theory and practice of psychosomatic medicine as applied to adults and children in all the medical specialties, and in contributions in psychophysiology and ecology. There will be one panel devoted to the adrenal cortex. Abstracts of contributions to this panel will be considered by the committee. Papers accepted for presentation at the meeting will be submitted to the editorial board of *Psychosomatic Medicine* for possible publication in that journal.

Material for consideration by the program committee should be sent, in duplicate, to Dr. Sydney G. Margolin, Chairman, 551 Madison Ave., New York 22.

GENERAL NEWS

Medical Research Grants Totaling $264,424 Announced by Lasdon Foundation.—Grants for medical research totaling $264,424 have been appropriated for 1952 by the Lasdon Foundation. More than a score of institutions here and abroad will share in the grants.

One of the largest individual grants was awarded to the Rheumatic Fever Institute of Northwestern University for studies on the influence of dietary factors and enzyme metabolism on the development and treatment of rheumatic fever. Another large grant was given the University College, School of Medicine of Dublin, Ireland, for chemotherapeutic research in the field of tuberculosis, under the direction of Dr. Vincent C. Barry.

The Foundation is a philanthropic organization established four years ago by the Lasdon brothers, William S., Milton S., Stanley S., and J. S., to support research exclusively in the fields of medicine and health, an interest stemming from the Lasdon family's long affiliation with the pharmaceutical industry and association with the Nepera Chemical Co., Inc., of Yonkers, N. Y.

Books

Physical Medicine and Rehabilitation for the Clinician. Edited by Frank H. Krusen, M.D. Price, $6.50. Pp. 371, with 96 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1951.

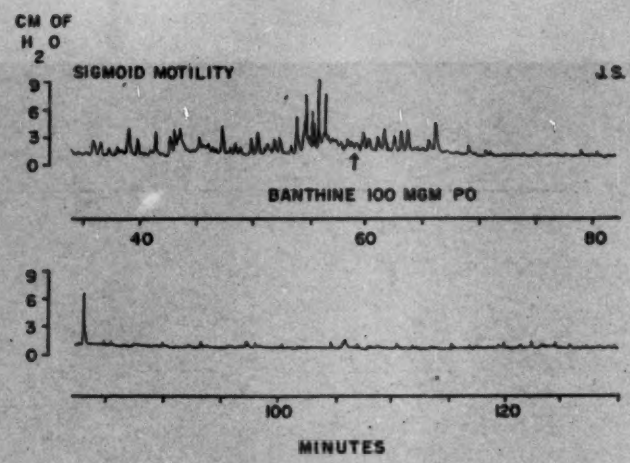
As indicated by the title and emphasized in the preface, this collection of chapters by more than 20 contributors is primarily for the medical student and clinician in fields other than physical medicine. The first two sections of the book present adequate details to enlighten the reader on the therapeutic and diagnostic applications of physical agents and procedures and to aid the clinician in understanding his responsibilities when enlisting the aid of the physical therapist in the management of his patients. The third section presents the clinical aspects of physical medicine and rehabilitation, particularly concerned with neurologic, musculoskeletal, and thoracic diseases. While the section is broad in scope, covering the social, psychosomatic, and diagnostic aspects of this group of diseases as related to physical medicine, some of the individual chapters are too brief to do more than present the problem and make the reader aware of its existence. The fourth section of the book is a brief review of the functional anatomy of the musculoskeletal system. This book can serve well as a desk reference for the clinician when he is confronted with problems involving physical medicine, but is not nor is it intended to be in any sense encyclopedic.

Infective Hepatitis: Studies in East Anglia During the Period 1943-47. By F. O. MacCallum, A. M. McFarlan, J. A. R. Miles, M. R. Pollock, and C. Wilson. Medical Research Council Special Report Series No. 273. Price, 4s. 6d. Pp. 144, with charts and tables. His Majesty's Stationery Office, Box 569, London, S. E. 1, 1951.

The investigation encompassed in this short monograph ended in 1947. Publication was postponed because much of the work already had been described in the medical press. Recently, however, it was decided to publish the material without revision and to give the factual results as first recorded.

This is an excellent and detailed reference work for students interested in virus hepatitis. A great part of it is concerned with epidemiologic studies. The clinical observations are still basically sound, but the biochemical observations, for the most part, have been altered considerably since the work herein reported was done. A great amount of hematologic study is recorded, and the chapter on experiments in transmission is basic reading for students of this disease.

The monograph would not particularly interest general practitioners or specialists in internal medicine but would be of value to those interested in gastroenterology.



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1. Kern, F., Jr.; Almy, T. P., and Stolk, N. J.: Effects of Certain Antispasmodic Drugs on the Intact Human Colon, with Special Reference to Banthine (β-Diethylaminoethyl Xanthene-9-Carboxylate Methobromide), Am. J. Med. *11*:67 (July) 1951.

2. Lepore, M. J.; Golden, R., and Flood, C. A.: Oral Banthine, an Effective Depressor of Gastrointestinal Motility, Gastroenterology *17*:551 (April) 1951.

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1. Humphreys, P., et al.: Angiology 3:1 (Feb.) 1952.
2. Plotz, M.: N.Y. State J. Med. 52:2012 (Aug. 15) 1952.

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1. Hutcheson, J. M.: Management of Cardiac Failure. *Virginia Med. Monthly*, 74:458, Oct., 1947.
2. Noth, P. H.: Pick's Disease: A Record of Eight Years' Treatment with Salyrgan, Ammonium Nitrate, and Abdominal Paracentesis. *Proc. Staff Meet. Mayo Clin.*, 12:513, Aug. 18, 1937.

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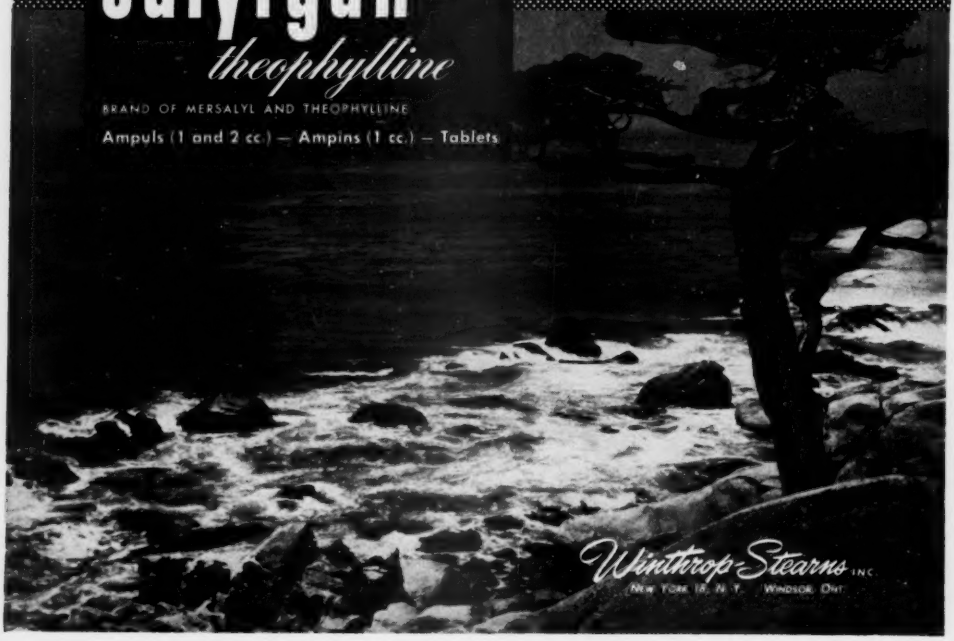
BRAND OF MERSALYL AND THEOPHYLLINE

Ampuls (1 and 2 cc.) — Ampins (1 cc.) — Tablets



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